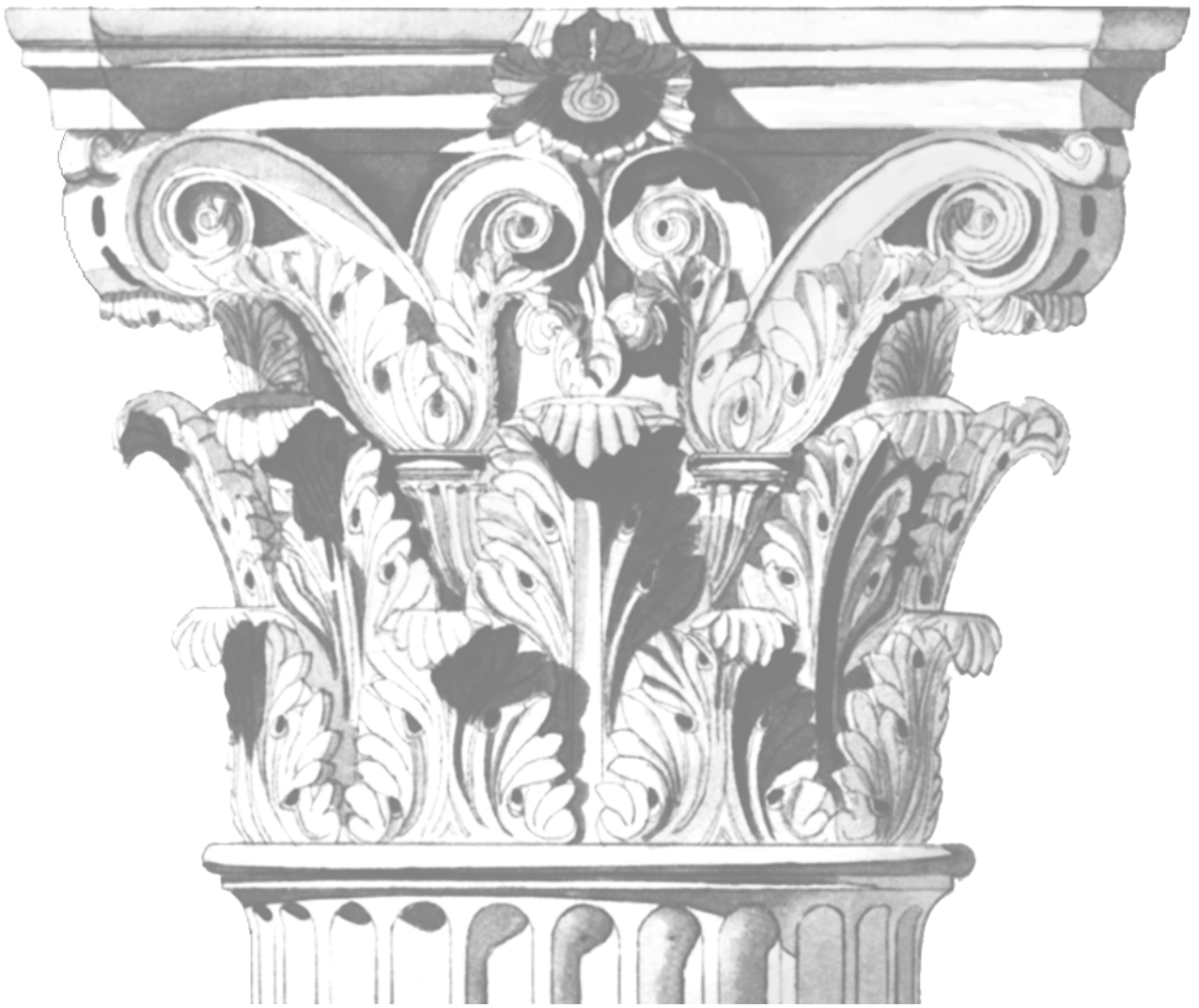
INFLAMMATORY RESPONSE FOLLOWING ABDOMINAL SURGERY AND ITS MODULATION BY RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR (RHG-CSF, FILGRASTIM)

HEIKKI WIIK

Department of Surgery and
Department of Medical Microbiology,
University of Oulu and
Department of Infection Control,
University Hospital of Oulu

OULU 2002



HEIKKI WIIK

INFLAMMATORY RESPONSE FOLLOWING ABDOMINAL SURGERY AND ITS MODULATION BY RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR (RHG-CSF, FILGRASTIM)

Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium 1 of the University Hospital of Oulu, on November 1st, 2002, at 12 noon.

OULUN YLIOPISTO, OULU 2002

Supervised by
Docent Kari Haukipuro
Docent Hannu Syrjälä

Reviewed by
Docent Juha Perttilä
Docent Heikki Repo

ISBN 951-42-6847-4 (URL: http://herkules.oulu.fi/isbn951426847 4/)

ALSO AVAILABLE IN PRINTED FORMAT
Acta Univ. Oul. D 699, 2002
ISBN 951-42-6846-6
ISSN 0355-3221 (URL: http://herkules.oulu.fi/issn03553221/)

OULU UNIVERSITY PRESS
OULU 2002

Wiik, Heikki, Inflammatory response following abdominal surgery and its modulation by recombinant human granulocyte colony-stimulating factor (rhG-CSF, filgrastim)

Department of Surgery, University of Oulu, P.O.Box 5000, FIN-90014 University of Oulu, Finland, Department of Medical Microbiology, University of Oulu, P.O.Box 5000, FIN-90014 University of Oulu, Finland, Department of Infection Control, University Hospital of Oulu, P.O.Box 22, FIN-90029 OYS, Finland

Oulu, Finland
2002

Abstract

The effects of perioperative filgrastim (rhG-CSF) and surgery *per se* on the postoperative acute phase reaction were studied by assessing leukocyte functions, cytokine levels and tenascin-C (Tn-C) and procollagen propeptide (PINP, PIIINP) concentrations in different body fluid compartments in patients undergoing gastrointestinal surgery.

Thirty consecutive patients were randomized to receive either filgrastim or placebo for five days, starting 12 hours before colorectal surgery. Filgrastim treatment led to marked neutrophilia with decreased neutrophil migration in peripheral blood but not in peritoneal fluid 48 hours postoperatively. Neutrophil phagocytosis and bacterial killing did not differ between the groups. Filgrastim caused increased postoperative expression of neutrophil CD11b/CD18 in blood but not in peritoneal fluid or wound fluid. CD11b/CD18 expression was higher in both wound fluid and peritoneal fluid than in blood in the placebo group. The expression of neutrophil CD62L was higher in blood than in peritoneal fluid or wound fluid in both groups. The serum concentration of interleukin (IL)-8 was lower in the filgrastim group 5 hours postoperatively. The concentrations of IL-1β, IL-6, transforming growth factor (TGF)-β and IL-10 did not differ between the groups. The cytokine levels were markedly higher locally in the wound and in the peritoneal cavity compared to circulating blood. No adverse events attributable to filgrastim were seen.

Leukocyte counts, neutrophil and monocyte functions and the levels of IL-6, IL-8 and granulocyte colony-stimulating factor (G-CSF) were measured from 18 patients before and after colorectal surgery. Surgery caused an increase in neutrophil and monocyte counts along with lymphocytopenia. Neutrophil phagocytosis was decreased 4 and 24 hours postoperatively, but normalized after that. A distinct systemic cytokine response was seen postoperatively.

In a study with 24 patients, Tn-C concentration increased in wound fluid during the first postoperative week after abdominal surgery. The Tn-C level was markedly higher in wound fluid than in serum.

Keywords: acute phase response, cytokine, leukocyte, wound healing

To my family

Acknowledgements

The present study was carried out in the Department of Surgery in co-operation with the Department of Infection Contro, and Clinical Chemistry, Oulu University Hospital, the Department of Medical Microbiology, University of Oulu and National Public Health Institute, Oulu, during 1996–2002.

I am grateful to Professor Matti Kairaluoma, M.D., Ph.D., former Head of the Department of Surgery for giving me the opportunity to start this work.

I also express my thanks to Professor Tatu Juvonen, M.D., Ph.D., present Head of the Department of Surgery for co-operation.

I owe my warmest thanks to my supervisor, Docent Kari Haukipuro, M.D., Ph.D., Head of the Division of Anaesthesiology, Surgery and Neurosurgery, for introducing me to scientific work and to my other supervisor, Docent Hannu Syrjälä, M.D., Ph.D., for keeping me there long enough to complete this thesis.

I thank my excellent co-authors Docent Riitta Karttunen, M.D., Ph.D., Docent Sylvi Silvennoinen-Kassinen, M.D., Ph.D., Docent Heljä-Marja Surcel, Ph.D., and Professor Juha Risteli, M.D., Ph.D., for efficient teamwork.

I am grateful to my collaborators Jouko Laurila, M.D., Kari Luukkonen, M.D., Aini Bloigu, B.Sc., Päivi Mertaniemi, Ph.D., and Riitta Kaarteenaho-Wiik, M.D., Ph.D.

I express my special appreciation to Docent Heikki Repo, M.D., Ph.D., and Docent Juha Perttilä, M.D., Ph.D., for reviewing the present manuscript and providing constructive comments and criticism.

I wish to thank Pasi Ohtonen, M.Sc., for sharing his knowledge on biostatistics.

I owe my special thanks to Mrs. Pirjo Kiirikki, Mrs. Marja-Leena Hannus, Mrs. Johanna Perälä, and Mrs Elsi Saarenpää for technical assistance.

I wish to express my warmest thanks to Maija Pesola, M.D., Ph.D., my colleague as an administrative physician, for indispensable help during these years.

I also want to give my warmest thanks to my long-standing colleague, friend and collaborator Juha Saarnio, M.D., Ph.D., for his unfailing backup in clinical work.

I owe my thanks to all my colleagues and staff of the Department of Surgery.

I thank Sirkka-Liisa Leinonen, Lic.Phil., for prompt and excellent language revision.

I wish to express my sincere thanks to the Wenezian Brothers: Juha Koskenkari, M.D., Pekka Kunelius, M.D., Ph.D., Pasi Kurtti, M.D., Tero Rautio, M.D., Ph.D., Jorma Ryhänen, M.D., Ph.D., Juha Veijola, M.D. and Juha Välimäki, M.D., Ph.D., for offering me a continual opportunity for regression in good company.

I also want to thank the fabulous Cool Operator rock'n'roll group along with Heikki Takala, M.D., and Auvo Hietaharju for unforgettable gigs around Finland.

I am deeply grateful to my parents Sirkka and Niilo Wiik for their love and excellent genetic heritage.

Finally, I owe my deepest gratitude to my wife Riitta and to our children Vili, Akseli and Aliisa. You are the sunshine of my life!

This work was supported by grants from Amgen Ab and Finnish Medical Society Duodecim.

Oulu, September 2002 Heikki Wiik

Abbreviations

CD	Cluster of differentiation
CD 62E	Endothelial E-selectin
CD 62L	Leukocyte L-selectin
CD 62P	Platelet P-selectin
ECM	Extracellular matrix
FMLP	N-formyl-methionyl-leucyl-phenylalanine
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HLA	Human leukocyte antigen
ICAM	Intercellular adhesion molecule
IFN	Interferon
IL	Interleukin
IL-1Ra	Interleukin-1 receptor antagonist
LPS	Lipopolysaccharide
LT	Leukotriene
M-CSF	Macrophage colony-stimulating factor
MHC	Major histocompatibility complex
NADPH	Nicotinamide adenine dinucleotide phosphate
PINP	Aminoterminal propeptide of type I procollagen
PIIINP	Aminoterminal propeptide of type III procollagen
rhG-CSF	Recombinant human granulocyte colony-stimulating factor
sIL-6R	Soluble interleukin-6 receptor
sTNFR-1	Soluble tumour necrosis factor receptor-1
TGF	Transforming growth factor
Tn	Tenascin
TNF	Tumour necrosis factor

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals.

I Wiik HT, Syrjälä HP, Silvennoinen-Kassinen SH, Laurila JJ & Haukipuro KA (1999) Use of recombinant human granulocyte-colony stimulating factor in colorectal surgery. Eur J Clin Microbiol Infect Dis 18: 819–822.

II Wiik H, Syrjälä H, Karttunen R, Bloigu A & Haukipuro K (2001) Neutrophil adhesion molecules in colorectal surgery: effect of Filgrastim given perioperatively. Eur J Surg 167: 700–704.

III Wiik H, Karttunen R, Haukipuro K & Syrjälä H (2001) Maximal local and minimal systemic cytokine response to colorectal surgery: the influence of perioperative Filgrastim. Cytokine 14: 188–192.

IV Wiik HT, Haukipuro KA, Karttunen RA, Surcel H-M, Saarnio JM, Luukkonen KP & Syrjälä HP. Transient decrease of neutrophil phagocytic capacity after colorectal surgery. (submitted for publication).

V Wiik HT, Kaarteenaho-Wiik RL, Mertaniemi PE, Risteli JP, Syrjälä HP & Haukipuro KA. Increased postoperative concentrations of Tenascin-C in wound fluid. (submitted for publication).

Contents

1 Introduction

Postoperative infectious complications are a major problem in surgery. Pathogenicity of microbes, environmental factors and host defence mechanisms are the three basic determinants of the infection process.

The factors contributing to the high infection rate in gastrointestinal surgery include intraoperative bacterial contamination, the abdominal wound site itself, wound class and the length of operation (Cruse & Foord 1980, Haley *et al.* 1985, Claesson & Holmlund 1988).

In elective colorectal surgery, bacterial contamination of the operative field is the most powerful predictor of postoperative infection (Claesson & Holmlund 1988). Neutrophils have a central role in eliminating bacteria from the wound and the peritoneal cavity. The suggested effects of surgery on neutrophil functions are contradictory, with both impairment and enhancement reported (Shigemitsu *et al.* 1992, Oka *et al.* 1994, Jensen *et al.* 1995). The studies addressing these changes have involved heterogeneous patient populations, sampling techniques and research methods, thus making it difficult to draw definitive conclusions on the topic.

Surgical patients may also have underlying conditions, such as high age, diabetes mellitus, alcoholism or a neoplasm, which impair the neutrophil phagocytic capacity (Esparza *et al.* 1996, Bagdade *et al.* 1974, Rajkovic & Williams 1986, Wiezer *et al.* 1999). Moreover, in gastric cancer patients, defects in superoxide generation by neutrophils have been described (Arii *et al.* 2000).

In addition, the local circumstances in the wound may be unfavourable for the elimination of pathogens postoperatively. The oxidative killing of neutrophils depends on the partial pressure of oxygen, and the hypoxic environment found in wounds may thus impede their function (Allen *et al.* 1997, Hopf *et al.* 1997).

Prophylactic antibiotics have been very successful in reducing infection-related morbidity and mortality in surgery (Cainzos 1998). Some new techniques, such as warming the patient during the operation and the perioperative use of supplemental oxygen, have also proven useful (Kurz *et al.* 1996, Greif *et al.* 2000, Melling *et al.* 2001). Lately, the use of immune-enhancing nutritional support preoperatively has yielded promising results (Tepaske *et al.* 2001).

Recently, a new range of agents have been identified and become available that are capable of stimulating and regulating host defence systems. These glycoproteins, whose systems of regulation are thus far poorly understood, are called cytokines, and some of them are growth factors. Granulocyte-colony stimulating factor (G-CSF), a glycoprotein that regulates the proliferation and differentiation of haematopoietic precursor cells also increases neutrophil functions, such as phagocytosis and killing (Fabian *et al.* 1991, Roilides *et al.* 1991, Lieschke & Burgess 1992,). Filgrastim is the recombinant human form of G-CSF (rhG-CSF).

The purpose of the present investigation was to assess the systemic and local effects of perioperative filgrastim in colorectal surgery. Secondly, the effect of colorectal surgery *per se* on leukocytes and cytokines postoperatively was assessed. Thirdly, the synthesis of certain extracellular matrix proteins (PINP, PIIINP, Tn-C) closely related to the acute inflammatory response of wound healing was studied.

2 Review of the literature

2.1 Pathophysiology of acute inflammation

2.1.1 General

An inflammatory cascade is initiated in several situations, including infection, trauma, surgery, burns, tissue infarction and advanced cancer (Dinarello 1997, Gabay & Kushner 1999).

Locally, the acute inflammatory response includes capillary vessel vasodilatation (congestion), exudation of plasma proteins (oedema), leukocyte adherence to endothelium, chemoattraction and local activation of leukocytes, release of numerous mediators, elimination of foreign substances (phagocytosis), elimination of recruited cells (apoptosis) and healing of tissue (Cavaillon & Duff 1999). The main components of the acute inflammatory response are cytokines, acute-phase proteins and leukocytes (Guillou 1995, Foëx & Shelly 1996, Gabay & Kushner 1999).

The systemic response following local inflammation is known as the acute-phase response (Fig. 1), which is marked by fever, increased synthesis of hormones, such as adrenocorticotropic hormone (ACTH) and hydrocortisone, increased production of white blood cells and production of acute-phase proteins in the liver (Goldsby *et al.* 2001). The production of acute-phase proteins is stimulated by the inflammation-associated cytokines, interleukin (IL)-1β, tumour necrosis factor (TNF)-α, IL-6, interferon (IFN)-γ, transforming growth factor (TGF)-β and IL-8, which are secreted mainly by activated tissue macrophages (Gabay & Kushner 1999). By definition, an acute-phase protein is a protein with an increase (positive acute-phase protein, e.g. C-reactive protein, CRP) or a decrease (negative acute-phase protein, e.g. albumin) in plasma concentration by at least 25 percent during an inflammatory disorder (Morley & Kushner 1982). An excessive uncontrolled inflammation is clinically identified as a systemic inflammatory response syndrome (SIRS) (Davies & Hagen 1997).

Neutrophils and monocyte/macrophages of the innate immune system are the main effector cells during acute inflammation (Ganz 1993, Smith 1994).

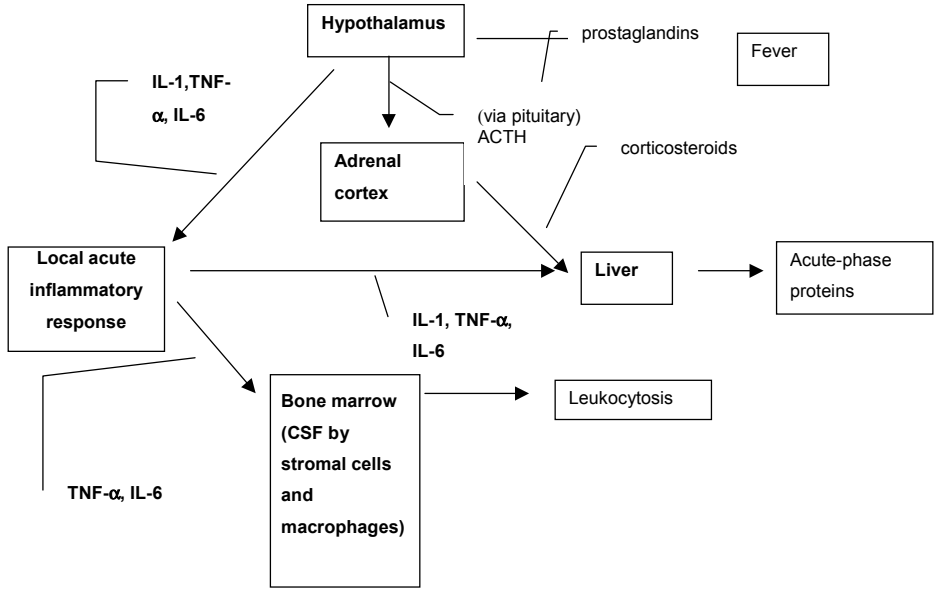


Fig. 1. Overview of the systemic acute-phase response. The data for this figure have been obtained from Goldsby *et al.* 2001.

2.1.2 Leukocyte-tissue interaction

Leukocytes, such as neutrophils, continuously patrol the vasculature, monitoring for signals of bacterial infection or inflammation. Substances released from pathogens (e.g. lipopolysaccharide, LPS) and tissue damage (e.g. TNF-α) upregulate the expression of adhesion molecules on vascular endothelium, and this, in turn, initiates the extravasation of leukocytes to the inflamed area (Delves & Roitt 2000). Leukocyte extravasation from blood into tissues involves several steps called random contact, rolling, sticking, diapedesis and chemotaxis (Fig. 2; Carlos & Harlan 1994).

When the leukocytes approach the post-capillary venules in areas of sub-endothelial inflammation, the initial random contact changes into rolling, which can be described as low-affinity adhesive interaction between leukocytes and the endothelium and defined as leukocyte movement through vessels at a rate lower than that of red blood cells (Granger & Kubes 1994). Rolling is mediated by the selectin family of adhesion molecules (Lawrence & Springer 1991, Lasky 1992). The selectin family comprises of three different proteins named according to the place where they were first discovered: endothelial E-selectin (CD 62E), platelet P-selectin (CD 62P) and leukocyte L-selectin (CD 62L). Both E- and P-selectins are also expressed by endothelial cells, but L-selectin is found only on leukocytes. (Carlos & Harlan 1994.) As leukocytes roll along the endothelium, there is continuous interaction and release between selectins and their counter-structural carbohydrate ligands, such as sialyl Lewis X, on leukocytes and

sulfated polysaccharides, such as fucoidan, on endothelial cells (Carlos & Harlan 1994.) Prior to firmer attachment, the L-selectin is shed from the surface of the leukocytes (Kuhns *et al.* 1995a).

The more stable adhesion, sticking, to the vessel wall is mediated by CD11/CD18 leukocyte adhesion molecules (β2 integrins) on leukocytes and intercellular adhesion molecules (ICAMs) on endothelial cells (Gahmberg 1997). The CD11/CD18 leukocyte adhesion molecule consists of three surface membrane heterodimeric glycoproteins named CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1, CR3) and CD11c/CD18 (p150,95). They all share a common β2 subunit (CD18) and have distinct α subunits: αL (CD11a), αM (CD11b) and αX (CD11c). (Arnaout 1990.)

After firm attachment, leukocytes start migrating across the endothelium via intercellular junctions into the subendothelial space. Both leukocyte and endothelial platelet-endothelial adhesion molecules-1 (PECAM-1, CD31) play an important role in this process, which is also referred to as diapedesis. (Carlos & Harlan 1994.)

Finally, leukocytes are attracted to the inflammatory sites through the production of chemoattractant mediators and chemokines, including N-formylated peptides, complement component C5a, leukotriene (LT) B4 and IL-8 (chemotaxis) (Bokoch 1995, Luster 1998).

2.1.3 Neutrophils at the site of inflammation

The lifespan of a neutrophil is approximately 14-16 days, of which 1-2 days are spent in tissues (Gordon 1994). The schematic kinetics of neutrophil production and lifespan is presented in Fig. 3.

The main task of neutrophils is the phagocytosis and killing of invading microorganisms (Tramont & Hoover 2000). Phagocytosis is accompanied by a prompt increase of oxygen consumption referred as “the respiratory burst”, in which superoxide anion is generated via membrane-bound NADPH oxidase, resulting in microbicidal oxidants, superoxide anion and hydrogen peroxide (Casimir & Teahan 1994). These, together with the contents of neutrophil granules, have an extensive killing capacity (Doherty & Janusz 1994, Smith 2001).The different types of neutrophil granules are listed in Table 1.

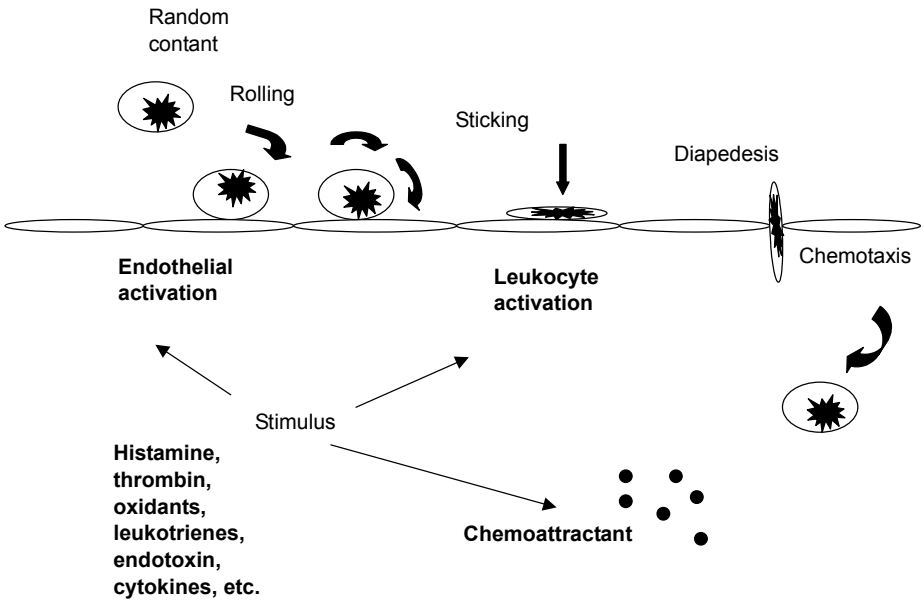


Fig. 2. The steps involved in leukocyte extravasation from blood into tissues. The data for this figure were obtained from Lasky 1992 and Carlos & Harlan 1994.

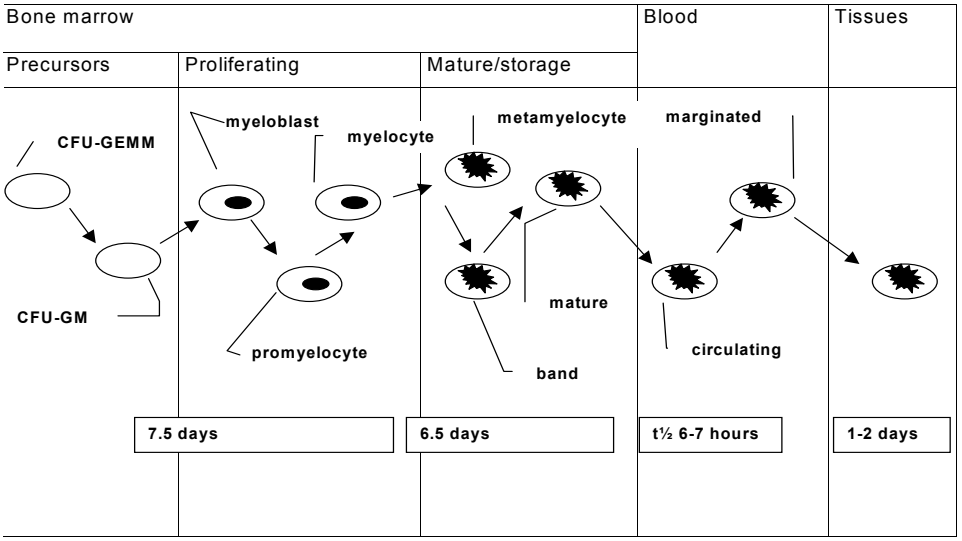


Fig. 3. The kinetics and lifespan of neutrophils. CFU-GEMM; colony-forming unit for granulocytes, erythrocytes and monocytes, CFU-GM; colony-forming unit for granulocytes and monocytes. Adapted and modified from Gordon 1994.

Table 1. Neutrophil granules and their most significant contents. Data for this table were obtained from Smith 2001.

Granule	Contents
Primary (azurophilic)	myeloperoxidase, elastase, cathepsin G, proteinase 3, lysozyme, α-mannosidase, β-glucuronidase, β-glycerophosphatase, sialidase
Secondary (specific)	lactoferrin, lysozyme, gelatinase, histaminase, sialidase
Tertiary	lysozyme, gelatinase, acetyltransferase
Secretory vesicles	alkaline phosphatase

Although these mechanisms have evolved to facilitate transit in tissues and the killing of bacteria, neutrophils also have a capacity to injure their host tissue as a 'side effect'. For example, the tissue injury associated with severe sepsis and multi-organ failure is mediated, in part, by the secretion of reactive oxygen intermediates and proteinases by adherent neutrophils in the microvasculature (Haslett *et al.* 1989, Okrent *et al.* 1990, Tanaka *et al.* 1991). However, it is assumed that the injurious effects mediated by neutrophils in acute inflammation are normally readily outweighed by local defense mechanisms (Haslett *et al.* 1989).

Neutrophils are eliminated by apoptosis, which is characterized by shrinkage of cells with condensation of chromatin (Wyllie *et al.* 1980). While normal leukocytes survive for less than 24 hours in the circulation before undergoing apoptosis, apoptosis is delayed in leukocytes migrating into inflammatory foci (Jimenez *et al.* 1997). Apoptotic cells are principally ingested by macrophages (Haslett & Henson 1996).

2.2 Acute inflammation triggered by surgery and anaesthesia

2.2.1 General

Surgical stress is due to stimuli caused by psychologic stress, tissue injury, intravascular volume redistribution, organ dysfunction and the sequlae of extirpative procedures and perioperative complications (Udelsman & Holbrook 1994). This makes it difficult to distinguish between the actual effects of different stimuli. Mere anaesthesia has minor effects on the immune functions, but inhibits the effects of surgery by reducing pain and maintaining homeostasis during operations (Salo 1992).

In surgery, a localized inflammatory response is advantageous, as it enhances immune defences and initiates wound healing. However, if the response is exaggerated by either a persistent tissue insult or a second insult, multiple organ dysfunction may develop (Demling *et al.* 1994).

2.2.2 Cytokine response to surgery

The cytokine response to elective surgery is basically similar but less intensive compared to infections or injury. The inflammatory cascade is initiated by the production of IL-1β and TNF-α followed by IL-6 and IL-8. (Damas *et al.* 1997). The inflammation is regulated by the balance between proinflammatory (IL-1β, TNF-α, IL-6, IL-8, IL-12, IL-18) and anti-inflammatory (TGF-β, IL-4, IL-10, IL-13) cytokines.

The cytokines known to be involved in elective surgery as well as their sources and main effects are shown in Table 2.

The systemic cytokine response to surgery has been studied and reviewed very actively (Lin *et al.* 1998). In contrast, only a few reports have addressed the cytokine concentrations at operative sites in humans, and these are summarized in Table 3.

Table 2. Cytokines released during controlled elective surgery with their principal sources and main effects. ↑; increase, ↓; decrease. The data presented in this table were obtained from Lin et al. 1998.

Cytokine	Source	Effect
TNF-α	Monocyte/macrophage, T-lymphocyte	↑ neutrophil release from bone marrow, ↑ neutrophil activation, migration, degranulation and superoxide production, ↑ monocyte/ macrophage differentiation, ↑ IL-6 induction, ↑ wound healing through increased endothelial procoagulant activity, leukocyte adhesion, vascular endothelial permeability, neovascularization, fibroblast proliferation and collagen synthesis
IL-1β	Monocyte/macrophage, T-lymphocyte, natural killer cell, endothelial cell, epithelial cell, keratinocyte, fibroblast, dendritic cell	↑ T-lymphocyte activation and proliferation, ↑ neutrophil release from bone marrow, ↑ neutrophil migration, ↑ monocyte/macrophage differentiation, ↓ pain perception through increased endorphin release and brain opiate-like receptors, ↑ IL-6 induction, ↑ wound healing
IL-2	T-lymphocyte	↑ overall immunocompetence, ↑ cytotoxic T-lymphocyte proliferation, ↑ reticuloendothelial system activity, ↑ gut barrier immunity
IL-6	Monocyte/macrophage, T-lymphocyte, fibroblast, endothelial cell	↑ fibroblast antiviral activity, ↑ lymphocyte differentiation, ↑ B-lymphocyte immunoglobulin production, ↑ hepatocyte acute-phase protein production, ↑ prostaglandin production,
IL-8	Monocyte/macrophage, T-lymphocyte, endothelial cell, platelet	↑ chemotaxis of neutrophils, macrophages and lymphocytes
IL-10	B-lymphocyte, T-lymphocyte	↓ inflammatory cytokine synthesis by monocyte/macrophages and lymphocytes, modulates inflammatory activities of TNF-α, IL-1β, IL-6, IL-8 and IFN-γ
IL-12	Monocyte/macrophage, neutrophil, keratinocyte, dendritic cell	Stimulates T-lymphocytes, ↑ lymphocyte and natural killer cell production, ↑ B-lymphocyte immunoglobulin production, ↑ haematopoiesis,
IFN-γ	Monocyte/macrophage, T-lymphocyte, natural killer cell	↑ monocyte/macrophage and neutrophil activation against invading organisms, ↑ expression of MHC class I and II surface antigens, ↑ monocyte/macrophage oxidative and cytotoxic activity, overall lymphocyte proliferation, ↑ TNF-α and IL-1β activity
GM-CSF	T-lymphocyte, fibroblast, endothelial cell, stromal cell	↑ myeloproliferation of monocytes, neutrophils and eosinophils, ↑ chemotaxis of neutrophils and monocyte/macrophages, ↑ cytokine production by monocyte/macrophages

Table 3. Studies examining cytokine concentrations locally at the operative site. PF; peritoneal fluid, WF; wound fluid, DR; drainage fluid, →; positive correlation.

Reference	Sample	Type of surgery (no. of patients)	Main results
Tsukada *et al.* 1993	PF	Abdominal surgery (49)	IL-1β, TNF-α, IL-6 → length of operation; IL-1β, TNF-α → peritoneal bacterial count; IL-1β → operative blood loss
Tsukada *et al.* 1994	PF	Abdominal surgery (27)	IL-6, IL-8 → length of operation and operative blood loss; IL-8 → neutrophil elastase activity
Ono *et al.* 1995	WF	Skin grafting (24)	High concentrations of PDGF, IL-6, TGF-α and TGF-β locally
Badia *et al.* 1996	PF	Abdominal surgery (12)	High concentrations of IL-1β, TNF-α and IL-6, during first 24 h postoperatively
Hisano *et al.* 1997	DF	Thoracoabdominal surgery (26)	High concentrations of IL-6 but not sIL-6R locally
Van Berge Henegouwen *et al.* 1998	PF	Abdominal surgery (12)	High concentrations of TNF-α, IL-6 and IL-10 locally. Second rise of peritoneal TNF-α in patients with complications
Krohn *et al.* 1999	DF	Major orthopedic surgery (8)	Different patterns of increase and decrease between Il-1β, IL-2, IL-6, TNF-α, IL-10, IL-1Ra, IL-6sR, sTNFR-1
Holzheimer & Steinmetz 2000	WF	Reduction mammoplasty (28)	High concentrations of IL-6, IL-8, sTNFR-1 and TGF-β locally

2.2.3 Leukocyte response to surgery

In general, responses to surgery involve changes in the distribution and function of members of the leukocyte family. These changes include early systemic leukocytosis characterized by an increase of neutrophils and monocytes and a decrease of lymphocytes and eosinophils (Salo 1992). In addition to the postoperative decrease of the total lymphocyte count, the distribution of lymphocytes has been either altered, demonstrating a fall in T-cell levels and a decrease in the ratio of CD4 to CD8 cells, or stable (Gupta 1987, Ryhänen *et al.* 1991). Lymphocyte functions have also been altered after surgical stress, including defects in antigen recognition, a proliferative response and a decrease in antibody production (Brandt 2001).

Macrophages have shown increased phagocytosis and microbicidal activity but decreased class II major histocompatibility complex (MHC) molecule expression after surgery (Neoptolemos *et al.* 1985, Ryhänen *et al.* 1991). Studies on monocytes from injured patients have identified an association between reduced postoperative HLA-DR expression and subsequent infection and mortality (Cabié *et al.* 1992).

Studies on the effect of surgery on the postoperative neutrophil response in human patients have been gathered in Table 4. Some of these studies have also explored monocyte functions. The list is not exhaustive. In addition to presenting actual results, it aims to illustrate the diversity of the trials that have addressed this topic during the past few decades.

Table 4. Studies on the neutrophil response to surgery. Symbols: ↑; increase, ↔; no change, ↓; decrease compared to preoperative values. NBT reduction; nitroblue tetrazolium reduction: represents oxidative metabolism. Chemiluminescence represents oxidative metabolism.

Reference	Type of surgery (no. of patients) and main results
Cullen *et al.* 1975	Elective surgery (18). Neutrophil and monocyte phagocytosis ↓, NBT reduction ↓ after anaesthesia
Stanley *et al.* 1976	Elective orthopedic, gynaegologic, plastic or neurosurgical operations (43). Neutrophil chemotaxis ↓ after anaesthesia, but was restored by surgery
Bowers *et al.* 1977	Healthy kidney donors (25). Neutrophil phagocytosis ↔, killing ↔, chemotaxis ↓ 1 h, ↔ 1 d,↑ 2 d, adherence ↓ 1 h, ↔ 1 d, ↔ 2 d after surgery
Philip *et al.* 1980	Abdominal hysterectomy (12). Neutrophil colony-forming units in culture (CFUc) ↓ 1 d, ↑ 7 d, ↔ 15 d after surgery,
Moudgil *et al.* 1981	Elective plastic, orthopedic and neurosurgical operations (30). Neutrophil chemotaxis ↓ immediately, ↓ 24 h, and ↓ 48 h after surgery, and was restored 72 h after surgery
El-Maallem & Fletcher 1981	Abdominal hysterectomy (31). Neutrophil phagocytosis ↔, killing ↓ 24 h, ↓ 72 h, ↔ restored 8 d after surgery
Endler *et al.* 1982	Hip arthroplasty (14). Neutrophil chemotaxis ↓ 1 d, ↑ 3 d, ↔ 6 d after surgery
Van Dijk *et al.* 1982	”Major surgical procedures” (48). Neutrophil chemotaxis ↔, phagocytosis ↔, chemiluminescence ↔ 24 h, and 48 h after surgery
Davies *et al.* 1983	Abdominal hysterectomy (10). Neutrophil β-glucuronidase activity ↔ 4 h, ↓ 24 h, ↔ 5 d, lysozyme activity ↓ 4 h, ↓ 24 h, ↔ 5 d, B_{12}-binding capacity ↓ 4 h, 24 h, ↔ 5 d after surgery
Mealy *et al.* 1987	Elective general surgery (13). Neutrophil chemiluminescence ↓ after anaesthesia, ↑ 24 h after surgery
Perttilä *et al.* 1987	Elective major abdominal surgery (11). Neutrophil chemiluminescence ↔ 1-7 d, proportion of high-peroxidase-activity neutrophils ↑ 1d, ↑ 3-4 d, ↔ 6-7 d after surgery
Krausz *et al.* 1988	Elective surgical procedures (20). Neutrophil random migration and chemotaxis ↔ 24 h, thromboxane B_2-production ↓ 24 h after surgery
Salo *et al.* 1988	General surgery (17). Neutrophil chemiluminescence ↓ in patients with major postoperative infections
Utoh *et al.* 1988	Open heart surgery, abdominal surgery (28). Neutrophil superoxide production ↓, LTB4 production ↑, LTC4 production ↓ 1 d after surgery
Shigemitsu *et al.* 1992	Gastrointestinal surgery (50). Neutrophil intracellular killing ↓, superoxide anion production ↓ 1 d, myeloperoxidase activity ↑ 1-3 d after surgery
Wakefield *et al.* 1993	Elective major abdominal operations (28). Neutrophil hydrogen peroxide production ↔, CD11b ↑ 1 d after surgery (uncomplicated patients), hydrogen peroxide production ↑, CD11b ↑↑ 1 d after surgery (patients with subsequent sepsis)
Oka *et al.* 1994	Gastrointestinal surgery (14). Neutrophil attachment ↑, elastase-releasing capacity ↑ after surgery. In patients with complications the rise was long-lasting
Jensen *et al.* 1995	Open heart surgery (12). Neutrophil chemotaxis ↓ 1 d after surgery
Khan et al. 1995	Gynaecological surgery (60). Neutrophil phagocytosis ↔, NTB reduction ↔ 24 h after surgery. Anaesthesia-induced dose-dependent ↓ in phagocytosis and NTB reduction in minor/ moderate, but not in major surgery

Table 4. (Continued)

Reference	Type of surgery (no. of patients) and main results
Takala *et al.* 1996	Open heart surgery (21). Neutrophil CD11b ↑ 2-4 h, ↑ 24 h, ↔ 48 h, ↔ 72 h after surgery. Monocyte CD 11b ↔ 2-4 h, ↑ 24 h, ↑ 48 h , ↔ 72 h after surgery.
Høgevold *et al.* 1996	Total hip prosthesis (8). Neutrophil CD11b/CD18 ↔ after surgery, CD62L ↑ at the end of the operation, ↔ 20 h after surgery Monocyte CD11b/CD18 ↑ 20 h, ↑ 6 d after surgery, CD62L ↑ at the end of the operation, ↑ 20 h, ↑ 44h, ↔ 6 d after surgery
Barry *et al.* 1997	Elective major vascular or gastrointestinal operations (46). Patients termed "high responders" and "low responders" based on the distribution of preoperative values around the median. High responders: neutrophil respiratory burst ↓ 1 d, ↓ 2 d, ↓ 3 d, ↔ 5 d, CD11b ↓ 1 d, ↔ 2 d, ↔ 3d after surgery. Low responders: neutrophil respiratory burst ↑ 1 d, ↑ 3 d, ↔ 5 d, CD11b ↑ 1 d, ↔ 3 d, ↔ 5 d after surgery
Klava *et al.* 1997	Major abdominal operations for malignant disease (25). Neutrophil CD11b ↑ 1 d, ↑ 3 d, ↔ 6 d after surgery. CD 11b expression and adhesion were higher in patients who developed subsequent sepsis
Toft *et al.* 1998	Open-heart surgery (8), hysterectomy (8). Open-heart surgery: neutrophil oxidative burst ↓ perioperatively, ↓ 2 h, ↓ 3 h after aortic declamping. Hysterectomy: neutrophil oxidative burst ↔ 1 h, ↔ 2 h,↓ 3 h (abdominal surgery) after surgery.
Sietses *et al.* 2000	Fundoplication: conventional (8), laparoscopic (8). Conventional: neutrophil phagocytosis ↓ 2h, ↔ 1 d, ↔ 4 d after operation. Laparoscopic: neutrophil phagocytosis ↔ 2h, ↔ 1 d, ↔ 4 d after operation. Neutrophil oxidative burst higher in the conventional group 2 h after surgery. Neutrophil CD11b expression higher in the conventional group 4 d after surgery.

2.3 Hematopoiesis

Most mature blood cells live only for a short time and must be replaced continuously throughout their life. Blood cells originate from a self-renewing population of multipotential hemopoietic stem cells. These stem cells generate progenitor cells committed irreversibly to one or another of the various hemopoietic lineages. Progenitor cells, in turn, can each regenerate clones of lineage-restricted cells that mature into specialized cells. (Williams 2000.)

This process is regulated by hematopoietic growth factors, which can be divided into different categories based on the cell population affected by them. The granulopoietic group of hematopoietic growth factors includes granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF, CSF-1) and IL-5. (Bagby & Heinrich 2000.)

G-CSF, GM-CSF and M-CSF are also called colony-stimulating factors (CSFs). They are glycoprotein regulators able to control the proliferation and differentiation of granulocytes, monocyte-macrophages and certain related hemopoietic cells (Metcalf 1989). In this review, we will concentrate on G-CSF.

2.4 Granulocyte colony-stimulating factor (G-CSF)

2.4.1 General

G-CSF was identified by the ability of post-endotoxin-treated mouse serum to induce differentiation of cells in a murine myelomonocytic leukemia cell line, the differentiation-responsive (D+) subline WEHI-3B (Metcalf 1980, Burgess & Metcalf 1980). At first, it was named granulocyte-macrophage differentiation factor (GM-DF). The biochemical characteristics of this factor were described, and it was renamed granulocyte colony-stimulating factor (Nicola *et al.* 1983).

Human G-CSF was first purified to near homogeneity from the human bladder carcinoma cell line 5637 and after that from CHU-2 squamous cancer cells (Welte *et al.* 1985, Nomura *et al.* 1986). The gene for human G-CSF was initially cloned from 5637 cells and expressed in *Escherichia coli* (Souza *et al.* 1986). Additional molecular and biological characterization was done by Zsebo *et al.* 1986.

Human G-CSF is encoded by a single gene located on chromosome 17q 11-22 (Simmers *et al.* 1987). G-CSF is a single-chain polypeptide with a molecular weight of 20.000 daltons when the molecule is glycosylated (Nomura *et al.* 1986). The carbohydrate moiety is not required for receptor binding or the biological activity of the molecule, but probably increases the resistance to proteolysis (Nicola 1989).

The ability to produce G-CSF is characteristic of many cell types after appropriate stimulation. Monocytes are the most prominent source of it, but mesothelial cells, fibroblasts and endothelial cells can also produce it (Koeffler *et al.* 1987, Kaushansky *et al.* 1988, Vellenga *et al.* 1988, Zsebo *et al.* 1988). In addition, a variety of tumours have also been reported to produce G-CSF (Baba *et al.* 1995, Ichiishi *et al.* 2000). Production of G-CSF can be induced *in vitro* by TNF-α, IL-1, GM-CSF, IL-4 and bacterial LPS (Koeffler *et al.* 1987, Kaushansky *et al.* 1988, Vellenga *et al.* 1988, Oster *et al.* 1989, Wieser *et al.* 1989).

Production of G-CSF is highly regulated and not constitutive (Demetri & Griffin 1991). Circulating levels of G-CSF have only been found in 12% of healthy volunteers, but inductive stimulation may increase G-CSF concentrations markedly (Watari *et al.* 1988, Kawakami *et al.* 1990, Cebon *et al.* 1994).

Initial characterization of the human G-CSF receptor was reported by Nicola *et al*, and a further description of the biochemical and molecular nature of the G-CSF receptor was provided later (Nicola *et al.* 1985, Fukunaga *et al.* 1990, Layton *et al.* 2001). Receptors for G-CSF are present on precursors and mature neutrophilic granulocytes (300-1000 receptors on each), monocytes and platelets, but have not been found on erythroid, eosinophilic or lymphoid cells (Nagata & Fukunaga 1991, Shimoda *et al.* 1993, Boneberg *et al.* 2000). In addition, G-CSF receptors have been found on the surfaces of non-hematopoietic cells, including endothelial cells and small-cell lung cancer cells (Bussolino *et al.* 1989, Avalos *et al.* 1996).

2.4.2 In vitro and in vivo activities

The primary effects of G-CSF on normal hematopoietic cells are limited to cells of the neutrophil lineage (Demetri & Griffin 1991).

At the myeloid progenitor-cell level, G-CSF stimulates the growth of neutrophil granulocyte precursors (colony-forming units granulocyte, CFU-G) (Welte *et al.* 1985, Souza *et al.* 1986, Zsebo *et al.* 1986).

On mature, i.e. postmitotic, neutrophils, G-CSF has been shown to have several effects. Some of these effects on neutrophil function are direct and do not require any other stimuli. The direct effects of G-CSF on neutrophil function include enhanced survival (Begley *et al.* 1986), inhibition of apoptosis (Hu & Yasui 1997), adherence to synthetic fibers (Yuo *et al.* 1989, Okada *et al.* 1990) and altered phenotype and surface molecule expression (Yong & Linch 1992, Kerst *et al.* 1993, Ohsaka & Saionji 1998, Zarco *et al.* 1999).

Many effects are indirect and require secondary stimuli, such as chemotactic factors, to be fully expressed. Among the indirect effects of G-CSF on neutrophil function are enhanced antibody-dependent cell-mediated cytotoxicity (Inoue *et al.* 1994, Bober *et al.* 1995) and respiratory burst (Avalos *et al.* 1990, Yuo *et al.* 1990, Yoshino *et al.* 1991), stimulation of arachinodic acid release (Avalos *et al.* 1990) and increased degranulation (de Haas *et al.* 1994).

G-CSF has been shown to enhance the phagocytic and bactericidal activity of neutrophils (Roilides *et al.* 1991, Bober *et al.* 1995). Studies concerning the effect of G-CSF on neutrophil chemotaxis have yielded opposite findings with enhancement and impairment (Wang *et al.* 1988, Azzara *et al.* 1996).

The results of neutrophil function tests may vary, however, depending on whether the effects are examined *in vitro* or *in vivo*, the particular assay conditions and the subject's clinical condition (Pitrak 1997).

In addition, G-CSF-treated neutrophils have shown increased IL-8 receptor mRNA expression and enhancement of labelled IL-8 binding to the cell surface. This upregulation correlated with the increased chemotactic activity of G-CSF-treated neutrophils. (Lloyd *et al.* 1995.)

Endothelial cells have specific receptors for G-CSF (Bussolino *et al.* 1989). Accordingly, G-CSF has been shown to induce endothelial cell functions related to angiogenesis. These functions include the stimulation of proliferation, migration and release of proteolytic enzymes by endothelial cells (Bussolino *et al.* 1989, 1991). G-CSF downregulates the intercellular adhesion molecule 1 (ICAM-1) on the surface of endothelial cells (Eissner *et al.* 1997). However, G-CSF has been shown to increase neutrophil migration across the vascular endothelium (Yong 1996).

2.4.3 Recombinant forms (rhG-CSFs)

Three types of rhG-CSF are commercially available. These are non-glycosylated filgrastim, glycosylated lenograstim, and N-terminal mutated nartograstim, of which the latter is not available in Europe. Although there are some small differences in the pharmacokinetics of the three rhG-CSFs, their pharmacodynamics seem identical (Tanaka

et al. 1997). The glycosylated rhG-CSF may be more effective in terms of leukocyte mobilization and induction of cytotoxicity (Watts *et al.* 1997, Sakagami *et al.* 2000), but the clinical effects of filgrastim and lenograstim on neutropenia appear to be identical (Bönig *et al.* 2001). Recently, a new G-CSF molecule (PEG-r-metHu-G-CSF9) with a longer biologically active half-life has been developed (van der Auwera *et al.* 2001, Lord *et al.* 2001).

In phase I and II studies, rhG-CSF was shown to increase the concentration of circulating neutrophils in a dose-dependent manner, regardless of the route of administration (Gabrilove *et al.* 1988, Morstyn *et al.* 1988, 1989, Bronchud *et al.* 1989). However, the immediate effect of rhG-CSF was a decrease in the circulating neutrophil counts during the first 30 to 60 minutes followed by elevation above normal values within four hours (Morstyn *et al.* 1988, 1989, Bronchud *et al.* 1989, Borleffs *et al.* 1998). The reason behind this transient fall in neutrophils is not known, but it has been tentatively attributed to margination of neutrophils into endothelial cells (Morstyn *et al.* 1989). In all studies, the neutrophil levels returned to normal within 1 to 7 days after the withdrawal of rhG-CSF.

According to the findings, neutrophils produced during a proliferative response to rhG-CSF had normal or enhanced functional properties, as shown in assays of chemotaxis, phagocytosis and killing (Gabrilove *et al.* 1988, Glaspy *et al.* 1988, Fabian *et al.* 1991).

No changes in eosinophil and basophil granulocyte counts have been reported following the administration of rhG-CSF. Monocyte counts have also been unchanged at low doses, but a marked increase in monocytes has been noted following 30-100 μg of rhG-CSF administered as rapid intravenous infusion. A dose-independent increase in lymphocytes has been noted with daily intravenous administration of rhG-CSF. No consistent effects on hemoglobin, hematocrit or platelet counts have been noted in any study. (Gabrilove 1991.)

The pharmacokinetic studies of rhG-CSF have shown elimination by first-order kinetics without evidence of accumulation, the elimination half-life being 3.5 hours (Vincent *et al.* 1994). Filgrastim, r-metHuG-CSF (Neupogen®, Roche Ltd, Basel, Switzerland/ Amgen Inc, Thousand Oaks, CA), which was used in the present trial (I-III), is a hydrophobic protein composed of 175 amino acids with a molecular weight of 18.800 daltons. The protein is a single-chain polypeptide with two disulfide bonds, and it differs from the native protein in that it is not glycosylated and the N-terminal amino acid is methione, which is required for expression in *Escherichia coli*. (Osslund & Boone 1994.)

2.4.4 Clinical applications of rhG-CSF

Recombinant human G-CSF has been used in several clinical settings. Depending on the therapeutic indication and the country, the dosage of rhG-CSF ranges from 5 to 10μg/kg/day or 50 to 400 μg/m²/day (Frampton *et al.* 1994).

The indications include reduction of the incidence of infections in patients with non-myeloid malignancies on myelosuppressive standard-dose chemotherapy and both high-dose myeloablative and non-myeloablative chemotherapy (Crawford *et al.* 1991, Pettengell *et al.* 1992, Trillet-Lenoir *et al.* 1993, Demetri 1994, Mamounas *et al.* 1994, Beveridge *et al.* 1998, Toner *et al.* 1998, Fanning *et al.* 2000).

RhG-CSF has been used to decrease neutropenias due to congenital or acquired bone marrow failure. These include the myelodysplastic syndrome (Negrin *et al.* 1990), severe chronic neutropenia (Hammond *et al.* 1989, Boxer *et al.* 1992, Dale *et al.* 1993), aplastic anemia (Kojima *et al.* 1991) and drug-induced agranulocytosis (Sprikkelman *et al.* 1994).

RhG-CSF can shorten the period of neutropenia and reduce infectious complications in patients undergoing high-dose cytotoxic therapy with autologous bone marrow cell transplantation (Stahel *et al.* 1994).

In two recent studies, rhG-CSF treatment has failed to improve the survival of neutropenic patients in intensive care (Bouchama *et al.* 1999, Gruson *et al.* 2000).

Primary hematopoietic failure combined with the myelotoxicity of antiviral and anti-infective therapies often complicates the treatment of the acquired immunodeficiency syndrome (AIDS), and rhG-CSF has been used to ameliorate the problem (Kuritzkes 2000).

2.4.5 Adverse effects of rhG-CSF

Treatment with rhG-CSF has been well tolerated (Gabrilove 1991).

The predominant side effect has been medullary bone pain localized primarily to the lower back, pelvis and sternum (Gabrilove *et al.* 1988, Morstyn *et al.* 1989). In randomized trials, 15% to 39% of patients receiving approximately 5μg/kg/day have experienced this side effect, compared with 0% to 21% incidence in control patients (Crawford *et al.* 1991, Pettengell *et al.* 1992, Trillet-Lenoir *et al.* 1993, American Society of Clinical Oncology 1994, Maher *et al.* 1994). The pain has mostly occurred shortly after the initiation of rhG-CSF administration.

Other, infrequently reported side effects include exacerbation of a pre-existing inflammatory condition, e.g. eczema, psoriasis or vasculitis, exacerbation of osteoporosis, occasional rashes and allergic reactions, acute febrile neutrophilic dermatosis (Sweet syndrome), transient leukemia cutis in patients with chronic myeloid leukemia and rare injection site reactions (American Society of Clinical Oncology 1994, Vial & Descotes 1995). Moderate reductions in platelet counts with no hemorrhagic complications have been reported (Lindemann *et al.* 1989). Other deviations in laboratory values have included elevations in lactate dehydrogenase, uric acid and alkaline phosphatase (Gabrilove *et al.* 1988). Anti-G-CSF antibodies have not been reported (Crawford *et al.* 1991, Pettengell *et al.* 1992, Trillet-Lenoir 1993).

Doses of rhG-CSF higher than 100μg/kg/day have been given without dose-limiting toxicity (Amgen Inc. Data on file). A hematological study of healthy volunteers five years after rhG-CSF administration yielded no abnormal bone marrow findings (Sakamaki *et al.* 1995).

2.4.6 In infectious diseases

Treatment of infectious diseases in non-neutropenic patients is one of the possible new uses of colony-stimulating factors (Dale 1995, Stoltz *et al.* 1997).

The potential application of G-CSF to the treatment of infectious diseases is based on findings showing that circulating G-CSF increases with acute infectious diseases and after endotoxins (Kawakami *et al.* 1990, Kuhns *et al.* 1995b, Selig & Nothdurft 1995). The serum levels of G-CSF have been proportionate to the severity of infection and also a marker of bacterial origin (Kragsbjerg *et al.* 1995, Waring *et al.* 1995). Moreover, G-CSF levels have been higher in patients with Gram-negative than Gram-positive bacteraemia (Cebon *et al.* 1994). Persistent elevation of serum G-CSF after septic infection has predicted a poor outcome (Tanaka *et al.* 1996). Extremely high local G-CSF levels have been measured in the cerebrospinal fluid of patients with bacterial meningitis (Shimoda *et al.* 1991).

2.4.6.1 Experimental models

It has been shown that G-CSF-deficient mice lack adequate capability to resist infections (Lieschke *et al.* 1994, Zhan *et al.* 1998). Models of non-neutropenic animals support the idea that recombinant human G-CSF may augment host responses to infectious organisms in many settings, including severe burns (Mooney *et al.* 1988, Silver *et al.* 1989, Gamelli *et al.* 1995, Eaves-Pyles & Alexander 1996, Yalçin *et al.* 1997), sepsis (Toda *et al.* 1993, Patton *et al.* 1998), neonatal sepsis (Cairo *et al.* 1990, Novales *et al.* 1993), peritonitis (O'Reilly *et al.* 1992, Lorenz *et al.* 1994, Barsig *et al.* 1996, Dunne *et al.* 1996, Villa *et al.* 1998), colonic ischemia (Sullivan *et al.* 1993), pancreatitis (Çolak *et al.* 2001), hemorrhagic shock (Agalar *et al.* 1998) and pneumonia (Hebert *et al.* 1990, Abraham & Stevens 1992).

In the pneumonia model of cirrhotic rats, rhG-CSF treatment did not affect survival (Preheim *et al.* 1996). RhG-CSF has improved survival in experimentally induced infections with several extra- and intracellular bacteria, including *Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Pseudomonas aeruginosa* and *Mycobacterium avium* (Yasuda *et al.* 1990, Nelson *et al.* 1991, Lang *et al.* 1992, Serushago *et al.* 1992, Bermudez *et al.* 1998). Incubation of human neutrophils with rhG-CSF *in vitro* enhances both phagocytosis and killing of *Staphylococcus aureus*, which is a common pathogen found in surgical wound infections (Roilides *et al.* 1991). In addition, rhG-CSF has been shown to augment antifungal activities of neutrophils (Liles *et al.* 1997). In some studies, a synergistic effect of rhG-CSF and antibiotics has been demonstrated (Daschner *et al.* 1995, Kropec *et al.* 1995).

Although neutrophils are believed to play a central role in the pathogenesis of adult respiratory distress syndrome (Martin 1997), rhG-CSF has not had deleterious effects on systemic and pulmonary responses in experimental models (Kanazawa *et al.* 1992, Fink *et al.* 1993, Koizumi *et al.* 1993, Patton *et al.* 1998). In contrast, in an animal model of septic shock, rhG-CSF has been shown to protect mice via suppression of systemic TNF-α (Görgen *et al.* 1992, Lundblad *et al.* 1996). In an experimental model of colitis, rhG-CSF attenuated the inflammatory response (Hommes *et al.* 1996). In general, rhG-CSF seems to attenuate the release of proinflammatory cytokines in severe injury models and thus to modulate the overall immune response from pro- to anti-inflammatory balance (Hartung *et al.* 1995, Hartung 1999).

2.4.6.2 In humans

RhG-CSF treatment was associated with an improved clinical outcome in diabetic foot infections and opportunistic infections in HIV patients (Furumkin 1997, Gough *et al.* 1997, Kuritzkes 2000). In patients with acute traumatic brain injury or cerebral haemorrhage, rhG-CSF reduced the frequency of bacteremias but had no effect on mortality or other nosocomial infections (Heard *et al.* 1998). In patients with community-acquired pneumonia, rhG-CSF accelerated radiologic improvement and reduced serious complications, but did not affect the resolution of morbidity, mortality or the length of hospitalization (Nelson *et al.* 1998). In patients with multilobar community-acquired pneumonia, a trend towards reduced mortality among rhG-CSF-treated patients was reported (Nelson *et al.* 2000). In a study concerning patients with refractory chronic rhinosinusitis, no statistical significance was seen in the quality of life scores between a rhG-CSF group and a placebo group (van Agthoven *et al.* 2001).

In septic patients with neutropenia, rhG-CSF attenuated inflammatory responses without inducing tissue injury (Ishikawa *et al.* 1998). Downregulation of excessive systemic inflammatory responses by rhG-SF has been demonstrated in several studies (Pajkrt *et al.* 1997, Hartung 1998, Hartung *et al.* 1999, Boneberg *et al.* 2000). However, upregulation of both pro- and anti-inflammatory responses has also been shown in volunteers after rhG-CSF administration and when rhG-CSF pretreatment has been used before an inflammatory stimulus (Pollmächer *et al.* 1996a, 1996b, Xu *et al.* 1996). Accordingly, the timing of rhG-CSF-treatment in relation to the inflammatory stimulus, e.g. endotoxin challenge, may be critical (Pajkrt & van Deventer 1997).

2.4.7 In surgery

The conclusion from a small pilot study evaluating the effect of rhG-CSF on the prevention of post-operative wound infection was that the addition of rhG-CSF to standard therapy in patients undergoing radical vulvectomy is beneficial (van Lindert *et al.* 1995). In two recent studies on the prophylactic use of rhG-SF in surgery (neck dissection and esophagectomy for cancer), neutrophil funtions were enhanced in rhG-CSF-treated patients (Schäfer *et al.* 2000, Wenisch *et al.* 2000). A decreased incidence of postoperative infections in the rhG-CSF-treated patients was reported in all of the three above-mentioned trials. However, the series were too small to allow definite conclusions.

A large multicenter trial on the use of rhG-CSF in the prevention of postoperative infectious complications in patients with colorectal cancer has just been started (Bauhofer *et al.* 2001, Lorenz *et al.* 2001, Stinner *et al.* 2001).

RhG-CSF treatment has proved safe among patients in intensive care (Gross-Weege *et al.* 1997a, Pettilä *et al.* 2000, Wunderink *et al.* 2001). In surgical intensive care patients, rhG-CSF treatment has improved neutrophil functions and counterregulated the hyperactivation of the inflammatory response (Weiss *et al.* 1995, 1996, Gross-Weege *et al.* 1997b), and it is proposed that surgical intensive care patients with low or

undetectable G-CSF serum levels could benefit from rhG-CSF (Gross-Weege *et al.* 1997b). In esophageal surgery, too, rhG-CSF treatment has been shown to increase anti-inflammatory cytokines postoperatively (Hübel *et al.* 2000).

2.5 Wound healing and extracellular matrix

2.5.1 General principles

Normal wound healing consists of a cascade of cellular and biochemical events. The process is usually divided into three phases, which are the inflammatory phase, the proliferative phase and the remodelling phase (Lee & Doong 1993, Clark 1996).

The inflammatory phase begins almost immediately after the infliction of a wound. Its purpose is to remove damaged tissue and to restore immune defence mechanisms. A further goal of the inflammatory phase is the establishment of signals to guide the following stages. (Lee & Doong 1993.) When hemostasis has been established, neutrophils and monocytes begin to emigrate into the injured tissue to destroy pathogenic organisms and tissue debris (Clark 1996). As a whole, a properly organized inflammatory phase is a prerequisite for the next steps of wound healing.

The proliferative phase begins after the inflammation has subsided. It involves the proliferation of fibroblasts and endothelial and epidermal cells and the biosynthesis of extracellular matrix macromolecules, such as collagens and tenascin (Lee & Doong 1993).

During the remodelling phase, fibroblast proliferation ceases and the extracellular matrix matures to provide a connective tissue structure that is both strong and flexible (Lee & Doong 1993). Growth factors controlling the growth, differentiation and metabolism of cells during each of the three phases are summarized in Table 5.

Table 5. Growth factor activity in wound healing. TGF-α; transforming growth factor alpha, TGF-β; transforming growth factor beta, PDGF; platelet-derived growth factor, FGF; fibroblast growth factor, EGF; epidermal growth factor, IGF; insulin-like growth factor. The data for this table have been obtained from Steed 1997.

Growth factor	Cell source	Activity
TGF-α	platelets, macrophages, keratinocytes	activates neutrophils, fibroblast mitogen, stimulates angiogenesis
TGF-β	platelets, macrophages, lymphocytes	stimulates fibroblasia and angiogenesis, induces proliferation of many different cells
PDGF	platelets, macrophages, keratinocytes, endothelial cells	chemoattractant for neutrophils and fibroblasts, mitogen for smooth muscle cells and fibroblasts
FGF	macrophages, neural tissue	stimulates endothelial cell growth, mitogen for mesodermal- and neuroectodermal-derived cells
EGF	platelets, keratinocytes	mitogen for keratinocytes, endothelial cells and fibroblasts
IGF	liver	mitogen for fibroblasts, stimulates smooth muscle cells, lymphocytes and chondrocytes

2.5.2 Collagens

Collagens are essential components of most connective tissues. The main collagen types involved in wound healing are I and III. Initially, collagen III is deposited, and later on, it is gradually replaced by collagen I. (Eckes *et al.* 1996.) These collagens are synthesized in precursor forms, from which large polypeptides, called procollagen propeptides, are enzymatically cleaved off from both ends of the protein after secretion (Prockop *et al.* 1979). The postoperatively increasing concentrations of the procollagen propeptides I and III in wound fluid and serum reflect ongoing collagen synthesis (Haukipuro *et al.* 1991, 1992).

2.5.3 Tenascin

Tenascin is an extracellular matrix glycoprotein also known as myotendinous antigen, glioma mesenchymal ECM protein, hexabrachion, brachionectin, J1 and cytotactin (Erickson & Lightner 1988). It has an oligomeric structure containing domains homologous to epidermal growth factor (EGF), fibronectin type III repeat and the beta and gamma chains of fibrinogen, and it is thought to have a role in regulating cell proliferation, migration and differentiation (Lightner 1994).

Tenascin (Tn)-C is the most widely studied member of the tenascin family. It is upregulated during embryonic development, expressed in benign and malignant tumors, inflammation, fibrotic processes and wound healing and absent or restricted in most adult tissues (Mackie *et al.* 1988, Koukoulis *et al.* 1991, Kaarteenaho-Wiik *et al.* 1996, 2001). Recently, Tn-C serum levels have been shown to reflect the disease activity of inflammatory bowel disease (Riedl *et al.* 2001).

Several cytokines, including TGF-β, TNF-α, and IL-1, have been shown to induce an increased expression of Tn-C (Pearson *et al.* 1988, Rettig *et al.* 1994).

In normal human skin, Tn-C is present as a thin band in the papillary dermis immediately beneath the dermo-epidermal junction (Lightner *et al.* 1989). Healing wounds show markedly increased expression of Tn-C at all levels of skin (Mackie *et al.* 1988). Wound fibroblasts synthesize Tn-C, and in animal studies it appears 1 to 3 days after the injury and disappears within 10 to 21 days (Murakami *et al.* 1989, Chuong & Chen 1991, Luomanen & Virtanen 1992, Juhasz *et al.* 1993). Tn-C appears earlier in fetal wounds than in adults (Whitby *et al.* 1991). In humans, Tn-C is visualized 2-3 days after wounding and disappears within approximately 1.5 months (Betz *et al.* 1993, Latinjhouwers *et al.* 1996).

3 Aims of the study

1. To assess the effects and safety of perioperative filgrastim (rhG-CSF) in patients undergoing colorectal surgery (I)
2. To examine whether the postoperative expression levels of neutrophil adhesion molecules CD11b/CD18 (Mac-1) and CD62L (L-selectin) differ in peripheral blood, peritoneal fluid and wound fluid after colorectal surgery, and to analyze the effect of perioperative filgrastim on their expression (II)
3. To characterize the postoperative cytokine response after colorectal surgery simultaneously in peripheral blood, peritoneal fluid and wound fluid and to analyze the effect of perioperative filgrastim on this response (III)
4. To evaluate the effect of colorectal surgery on postoperative neutrophil and monocyte functions and cytokine levels (IV)
5. To assess the appearance of Tn-C in wound fluid and serum after abdominal operations and to compare it with the levels of PINP and PIIINP (V).

4 Material and methods

4.1 Clinical methods

The clinical work was carried out at the Department of Surgery, Oulu University Hospital. Altogether seventy-two patients were included in three trials. The study profiles are summarized in Table 6.

Table 6. Study profiles.

Trial	Surgery (n)	Study drug	Samples	Variables
1	colorectal surgery, randomized, blinded trial (30)	filgrastim vs. placebo	blood, peritoneal fluid, wound fluid	drug safety, leukocyte counts, neutrophil chemotaxis, phagocytosis and killing, neutrophil adhesion molecules (CD11b/CD18, CD 62L), CRP, IL-1β, TNF-α, IL-6, IL-8, TGF-β, IL-10
2	colorectal surgery (18)	none	blood	leukocyte counts, neutrophil and monocyte phagocytosis and respiratory burst, CRP, IL-6, IL-8, G-CSF
3	major abdominal surgery (24)	none	blood, wound fluid	PINP, PIIINP, Tn-C

4.2 Ethical considerations

In all trials, the study protocol was approved by the Ethical Committee of the Medical Faculty of the University of Oulu and in the first trial (I-III) also by The National Agency for Medicines, Finland. All trials were run according to the provisions of the Declaration of Helsinki. All patients gave written informed consent before entry into the trials.

4.3 Surgery and anaesthesia

The operations were done under standardized balanced anaesthesia (thiopentone, fentanyl, cisatracurium, isoflurane), and the patients were non-invasively monitored for blood pressure, electrocardiogram and peripheral blood oxygenation. A heating blanket was used to maintain normothermia during the operation (I-IV). Blood loss was compensated for by administering Ringer's acetate, and packed red cells were given, if necessary, to keep the haemoglobin concentration above 90 g/L. Postoperative pain was managed with continuous epidural fentanyl infusion (10 μg/mL) for three days (I-IV).

4.4 Study medication

For colorectal resections, the patients had preoperative oral bowel preparation (Klean-Prep, UCB, Brussels, Belgium), and the standard antibiotic prophylaxis of 2 g of ceftriaxone sodium hydrate and 1 g of metronidazole was given intravenously at the induction of anaesthesia. In gastric surgery, 1.5 g of cefuroxime was given. Subcutaneous low-molecular heparin (2500 IU dalteparin) was used as thrombosis prophylaxis.

In the first trial, the patients were randomized to receive either filgrastim (Roche Ltd, Basel, Switzerland / Amgen Inc, Thousand Oaks, CA) or placebo (I-III). The blinded study drug was administered at 5 μg/kg/day as subcutaneous injections for 5 days, starting 12 hours before the scheduled surgery. In addition to the study medication, the patients received any other necessary medication that they had been receiving before surgery.

4.5 Collection of samples

Blood samples were drawn from the cubital vein. Peritoneal fluid samples were harvested by puncturing the abdominal drain (Abdovac No. 18, Astra, Mölndal, Sweden). After the fascia had been closed, two silicone rubber tubes (Medical grade tubing No. 602-235, Dow Corning Corporation, Midland, Michigan, USA) were placed in the wound through two separate incisions to allow harvesting of wound fluid samples by puncturing the tube (I-III, V).

4.6 Leukocyte counts

The differential white cell counts were done using an automated cell counter (Technicon H 1, Bayer Corp., Tarrytown, NY). If the automated cell counter did not meet the counting criteria (showed the flaggings), then the sample was manually counted under a microscope (I-IV).

4.7 Neutrophil chemotaxis, phagocytosis and microbicidal activity

Blood samples for white cell counts were taken preoperatively and at 24 hours, after that daily until 6 days and, finally, at 30 days postoperatively (I).

Samples of blood, wound fluid and peritoneal fluid were taken at 48 hours postoperatively, and the tests to assess neutrophil functions were done immediately after sample collection. Chemotactic functions were tested from blood and peritoneal fluid, phagocytosis from blood, wound fluid and peritoneal fluid and bacterial killing from blood and peritoneal fluid. Granulocytes from blood samples were separated by Polymorphoprep (Nycomed, Oslo, Norway), those from wound fluid samples were separated by suspension in Hank's solution, and granulocytes from peritoneal fluid samples were separated by washing several times with Hank's solution before suspension. The neutrophil concentration was determined by differential count after the preparation of stained cytocentrifuge slides.

The chemotactic function of neutrophils was determined by Boyden´s chamber technique using the leading-front method (Wilkinson 1982). The migration chamber was divided by a membrane with a pore size of 3 µm into upper and lower compartments. The neutrophils were placed into the upper compartment in Hank´s solution at a concentration of 1 x 10^6/mL, while the lower compartment contained chemotactic stimulant. Five different chemoattractants were used: inactivated autologous serum, zymosan (Sigma, St. Louis, Missouri), activated autologous serum (2.5 mg/mL), casein (Sigma) in Hank´s solution (0.006 mg/mL), *E. coli* growth supernatant and *Staphylococcus aureus* growth supernatant. The chambers were incubated at 37 º C for 55 minutes.

The random movement/migration of neutrophils was measured in a chamber containing Hank's solution in both the upper and the lower compartments after 90 minutes of incubation. Chemokinesis (migration in a chemoattractant without a gradient) was determined in a chamber containing casein (0.006 mg/mL) in the upper and lower compartments after incubation at 37 º C for 55 minutes. The filters were removed after the incubations, fixed with ethanol, stained with haematoxylin (Merck, Darmstadt, Germany) and cleared with xylene. The migration distance of neutrophils from the starting point was measured by quantifying the distance migrated by the furthest two cells seen simultaneously in the microscope. The experiments were performed in triplicate, and five fields were examined per filter.

For the phagocytosis tests, *Staphylococcus epidermidis* was killed with formalin, washed with saline and added to a cell suspension containing 5 x 10^6 neutrophils/ mL at a ratio of five bacteria to one neutrophil in 25% Hank´s solution and inactivated autologous plasma (Ruutu & Kosunen 1972). The tubes were incubated, while being shaken, at 37º C for 6 minutes; smears were made and stained with Wright´s stain (Sigma). The percentage of neutrophils taking part in phagocytosis and the phagocytosis index (the average number of bacteria phagocytosed by one neutrophil) were determined microscopically by examining 100 cells.

For the tests on bactericidal activity, *Staphylococcus aureus* was grown in broth, washed with saline and added to a cell suspension containing 5 x 10^6 neutrophils/ mL and 10% pooled human serum in Hank's solution at a 1:1 ratio. The first samples were taken immediately after the mixing, and the cells were disrupted with Triton X-100; colony counts were made on blood agar (Ruutu & Kosunen 1972). The tubes were incubated with shaking at 37 º C, and new samples were taken after 1 and 2 hours and treated in the

same way. The bactericidal index was calculated by dividing the concentration of living bacteria at 1 and 2 hours by the initial concentration. The percentage of killed bacteria was recorded.

4.8 Neutrophil and monocyte phagocytosis and respiratory burst

Blood samples for white cell counts were taken preoperatively and at 4 hours, 6 hours, 12 hours, 24 hours and after that daily until 7 days postoperatively (IV).

Blood samples for the tests on neutrophil and monocyte phagocytosis and burst were taken preoperatively and at 4 hours, 24 hours, 2 days, 3 days and 7 days postoperatively. The tests were done immediately after sample collection.

The phagocytosis and respiratory burst of neutrophils and monocytes were measured using commercial test kits (Phagotest, Bursttest, Orpegen Pharma, Heidelberg, Germany) from whole blood samples. Phagotest measures the phagocytosis of fluorescein-labelled opsonized *Escherichia coli* provided by the manufacturer. The percentage and fluorescence intensity of neutrophils and monocytes that have ingested bacteria are measured with a flow cytometer, with both parameters reflecting the activity of cells but different aspects of it. The mean cellular fluorescence intensity unit (MFU) was taken as the result. In Bursttest, cells ingest unlabelled opsonized *Escherichia coli*, and the following respiratory burst is measured by the number of fluorescein-labelled reactive oxygen metabolites. The mean cellular fluorescence intensity unit (MFU) was taken as the result. Both tests were performed according to the recommended protocol.

A sample from a healthy control subject was tested in each series simultaneously with a patient sample to provide information on the accuracy of the test series. The coefficient of variation (CV) for the intra-assay variation was 5.1% for neutrophils and 6.6% for monocytes in Phagotest and 3.2% for neutrophils and 8.5% for monocytes in Bursttest (given by the manufacturer). The CV for day-to-day variation for a single sample was 7.8% for neutrophils and 8.7% for monocytes in Bursttest (tested in the laboratory). The respective figures for Phagotest were not estimated. The variation between individual samples in a test series was not estimated.

4.9 Neutrophil adhesion molecules

Samples of blood, peritoneal fluid and wound fluid for the measurement of neutrophil adhesion molecules were taken 48 hours postoperatively (II). Polymorphonuclear neutrophils were stained with monoclonal antibodies to CD11b, CD18, and CD62L (CD11b-PE, CD18-FITC, and CD62L-PE, Becton Dickinson, San Jose, CA, USA). The mean channel fluorescence intensities of each sample/staining were measured with a FACScan flow cytometer (Becton Dickinson).

4.10 Tests on cytokines and C-reactive protein (CRP)

In the first trial, the samples of blood, peritoneal fluid and wound fluid for cytokine assays were taken 5 and 24 hours after the operation (III). Cytokines were measured using commercial kits according to the manufacturer's instructions. Duoset ELISA kits (Genzyme, Cambridge, MA, USA) were used for TNF-α, IL-6, IL-8 and TGF-β. Pelikine-compact ELISA kits (CLB, Amsterdam, Netherlands) were used for IL-1β and IL-10. The lower detection limits were 15pg/mL for TNF-α, 4pg/mL for IL-6, 15pg/mL for IL-8, 62.5 pg/mL for TGF-β 2pg/mL for IL-1β and 1pg/mL for IL-10.

Samples for the measurement of CRP were taken preoperatively, at 24 hours and after that daily until 6 days and finally at 30 days postoperatively (I).

In the second trial, blood samples for cytokine assays and CRP were taken preoperatively and at 4 hours, 6 hours, 12 hours and 24 hours and after that daily until 7 days postoperatively (IV).

In the second trial, cytokine concentrations were determined by the enzyme immonoassay (ELISA) method using commercially available ELISA kits for IL-6, IL-8 (Duoset, Genzyme Diagnostics, Cambridge, USA) and G-CSF (Quantikine HS, R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. The lower detection limits were 4pg/mL for IL-6, 15 pg/mL for IL-8, and 0.4 pg/mL for G-CSF. CRP was determined with an automated Technicon H2 system (Tarrytown, NY) in our hospital laboratory.

4.11 Tests on procollagen propeptides and tenascin-C (Tn-C)

In the third trial (V), blood samples for the measurement of the serum concentrations of PINP, PIIINP and Tn-C were taken preoperatively and 24 hours, 2 days, 4 days, 7 days and 30 days postoperatively. Wound fluid samples for the measurement of the concentrations of PINP, PIIINP and Tn-C were taken daily for seven days postoperatively. All the samples were stored frozen at -20°C until analyzed. Before analysis, all the wound fluid samples were diluted 1:10 with phosphate-buffered saline, pH 7.2, containing 0.04% of Tween 20 (ICL Americas, Wilmington, DE). Further dilutions, when necessary, were also made in this buffer.

The aminoterminal propeptides of type I and III procollagens were analyzed using RIAs for human antigens (Orion Diagnostica, Finland). The levels of Tn-C in wound fluid and serum were quantified with an enzyme immunoassay based on two monoclonal antibodies specific to human tenascin, as described (Ylätupa *et al.* 1995).

4.12 Statistical methods

In all papers (I -V), summary measurements were expressed as means with standard deviation (SD), when normally distributed, or medians with the 25^{th} and 75^{th} percentiles (interquartile range, IQR), when skewed.

Groups were compared using Student's t-test (I, IV), Mann-Whitney U-test (II, IV) or Kruskal-Wallis test (III). The change over time was evaluated by Friedman's test (III, IV, V), Wilcoxon signed-rank test (II, III, IV) or Sign test (V). Analysis of Variance for repeated measurements was used to compare groups over repeated points of measurement (days) (IV). Two-tailed p-values were reported, and values of $p<0.05$ were considered statistically significant. Statistical evaluation was done using SPSS versions 7.5 (I, II), 9.0 (III, IV) and 10.0 (V) (SPSS Inc., Chicago, Il.).

5 Results

5.1 First trial

5.1.1 General

No withdrawals from the study occurred. One patient in the filgrastim group had peroperative cardiac arrest due to a vagal stimulus from packing the small bowel. The patient was resuscitated without postoperative complications. Another filgrastim-treated patients had a myocardial infarction and a mild congestive heart failure, which were treated conservatively. One placebo-treated patient had a clinical anastomotic leakage and required a re-operation. A wound infection developed after the second operation. One patient treated with filgrastim developed clinical signs of late postoperative infection (fever, increased CRP) three weeks after surgery. Despite thorough clinical and radiological examinations, the infection focus could not be localized. The patient was treated with intravenous antibiotics.

Wound healing estimated based on daily physical examinations did not differ between the groups. The course of postoperative temperature was similar in both patient groups.

There were no adverse events attributable to the treatment with filgrastim. However, the serum alkaline phosphatase level on day 6 after surgery was 317 (± 96) U/L in the filgrastim-treated group and 176 (± 77) U/L in the placebo-treated patients (*t*-test $p < 0.001$, 95 % CI 76 to 206). Thirty days after surgery, the serum alkaline phosphatase values had returned to the baseline level in both groups.

5.1.2 Leukocytes

Filgrastim had a distinct effect on the number and differential count of leukocytes: these haematologic changes are summarized in Fig. 4.

The median number of leukocytes (interquartile range) in peritoneal fluid 48 hours postoperatively was 33.2 (6.1-46.5) x 10^9/L in the filgrastim-treated group and 11.7 (2.8-32.5) x 10^9/L in the placebo-treated group (difference not statistically significant).

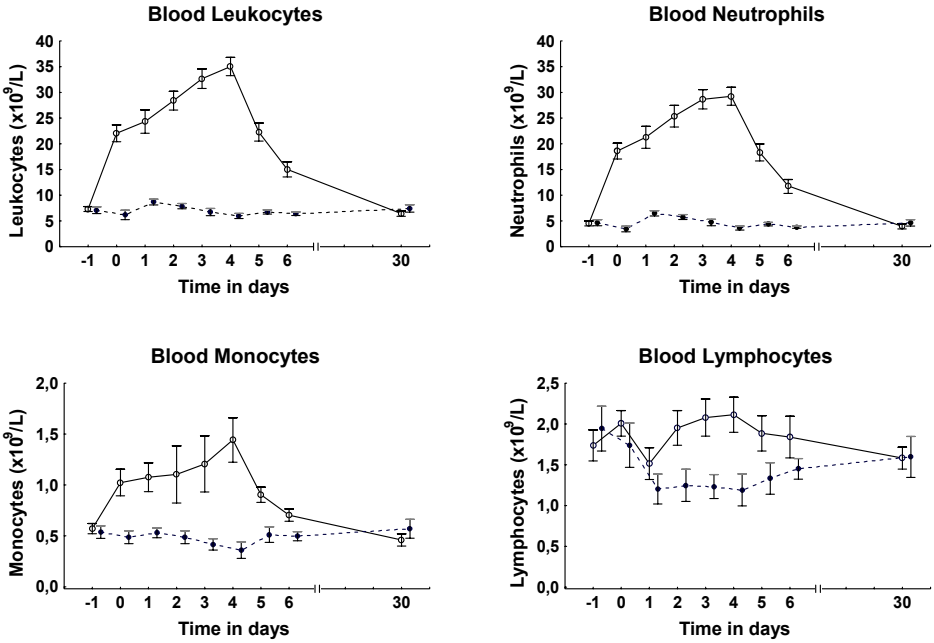


Fig. 4. The perioperative haematologic changes. Solid line; filgrastim group, dotted line; placebo group. The differences in total leukocyte, neutrophil and monocyte counts between the groups were statistically significant starting from the day of operation.

5.1.3 Neutrophil functions

The values for neutrophil random migration, chemokinesis and chemotaxis were higher in the placebo-treated group, when measured from circulating peripheral neutrophils. In peritoneal fluid, there was no difference between the groups.

There were no statistical differences in the percentage of phagocytosis or the phagocytosis index, nor in the percentage of bacterial killing of neutrophils between the filgrastim and placebo groups (Table 7).

Table 7. Neutrophil phagocytosis, phagocytosis index and bacterial killing. There were no significant differences between the groups. The numbers represent means and standard deviations.

		Filgrastim group	Placebo group
Phagocytosis (%)	Blood	78.1 (± 11.9)	72.4 (± 9.9)
	Peritoneal fluid	69.0 (± 13.8)	69.1 (± 12.3)
	Wound fluid	78.4 (± 5.0)	67.3 (± 9.0)
Phagocytosis index	Blood	2.9 (± 0.7)	2.6 (± 0.5)
	Peritoneal fluid	2.4 (± 0.7)	2.5 (± 0.7)
	Wound fluid	2.8 (± 0.2)	2.4 (± 0.6)
Killed bacteria (%) after 1 hour	Blood	26.8 (± 11.2)	32.8 (± 14.9)
	Peritoneal fluid	26.2 (± 14.4)	29.5 (± 14.2)
Killed bacteria (%) after 2 hours	Blood	56.0 (± 8.0)	50.6 (± 13.4)
	Peritoneal fluid	51.4 (± 6.5)	49.6 (± 8.8)

5.1.4 Neutrophil adhesion molecules

Filgrastim caused increased postoperative expression of neutrophil CD11b/CD18 in peripheral blood, but not in peritoneal fluid or wound fluid.

When intraindividual variation in CD11b/CD18 expression between the different body compartments was assessed in each group, it appeared that the expression was significantly higher in peritoneal fluid than in peripheral blood or wound fluid in both groups ($p < 0.05$, $p = 0.001$, and $p < 0.05$, $p = 0.01$ in the filgrastim and placebo groups, respectively). The difference between peripheral blood and wound fluid was not significant in the filgrastim group, but expression was significantly higher in wound fluid than in peripheral blood ($p< 0.01$) in the placebo-treated patients

There were no differences in the expression of neutrophil CD62L between the placebo and filgrastim groups in peripheral blood, wound fluid or peritoneal fluid.

When intraindividual variation in CD62L expression between the different body compartments was assessed in each group, expression appeared to be significantly higher in peripheral blood than in peritoneal fluid or wound fluid in both groups ($p< 0.01$, $p< 0.05$, and $p= 0.001$, $p=0.001$ in the filgrastim and placebo groups, respectively).

5.1.5 CRP and cytokine levels

The course of postoperative CRP values was as predicted and did not differ between the groups.

Except for the serum concentrations of IL-8, there were no statistically significant differences in cytokine concentrations between the filgrastim and placebo groups, and thus the comparisons between the fluids, with the exception of serum IL-8 concentrations, could be done with all the patients as one group.

Five hours postoperatively, the concentrations of all the measured cytokines, IL-1β, TNF-α, IL-6, IL-8, TGF-β and IL-10, were manyfold both in wound fluid and in peritoneal fluid compared with the concentrations in peripheral blood.

The differences between cytokine concentrations in wound fluid and peritoneal fluid were not so obvious. The levels of TGF-β and TNF-α were higher in wound fluid than in peritoneal fluid. On the contrary, the concentration of IL-10 was higher in peritoneal fluid than in wound fluid. The concentrations of Il-1β, IL-6, and IL-8 did not differ between the fluids at this time point.

Twenty-four hours postoperatively, the differences between peripheral blood and the local operative site still persisted (except IL-1β). At this time point, however, the levels of IL-6, IL-8 and TGF-β were higher in wound fluid than in peritoneal fluid. The concentrations of IL-1β, TNF-α and IL-10 showed no differences.

Also, either a statistically significant decrease or a clear tendency to decrease in the concentrations of cytokines in all fluids regardless of the medication group could be seen during the first twenty-four hours postoperatively. The only exception to this observation was the significant rise of IL-6 concentration in wound fluid in both the placebo- and the filgrastim-treated groups.

5.2 Second trial

5.2.1 Leukocytes

The patients had significantly increased total leukocyte counts postoperatively. The rise was due to the increase of neutrophils and monocytes and lasted for 6-7 days. In contrast, lymphocyte counts decreased immediately after the operation.

The mean neutrophil phagocytosis was lower 4 hours ($p=0.002$) and 24 hours ($p=0.036$) after the operation and returned to the preoperative level after that. The mean monocyte phagocytosis also showed a decreasing tendency 4 hours postoperatively, but the difference did not reach statistical significance. After that it increased, being significantly higher on the second ($p=0.004$) and the third ($p=0.014$) postoperative days.

There was also a decreasing tendency in the mean neutrophil respiratory burst 4 hours postoperatively. Correspondingly, the mean respiratory burst of monocytes increased slightly during the first three postoperative days.

5.2.2 CRP and cytokine levels

The values of CRP increased, starting from 12 hours postoperatively ($p<0.001$) and peaking at 2 days after surgery. After that, the values began to decrease, but did not reach the baseline level during the study period.

The cytokine response showed marked interindividual variation, and no differences of statistical significance were hence seen at any of the time points. The IL-6 concentrations increased slowly, being highest 4 days postoperatively. The IL-8 levels decreased for the first 12 hours. After that, the IL-8 concentrations started to rise, peaking at 4 days postoperatively.

The G-CSF levels showed a slight rise 4 hours postoperatively, then a decrease at 12 hours, and after that a slow rise with a peak on the fourth postoperative day.

5.3 Third trial

5.3.1 Procollagen propeptide concentrations

After the operation, the serum concentrations of PINP initially decreased and then started to increase. The highest value was measured on the 30th postoperative day. The serum concentrations of PIIINP showed a short drop on the first postoperative day and thereafter increased until the seventh postoperative day.

In wound fluid, the concentrations of PINP and PIIINP started to rise on the second postoperative day, reaching their peak values on the last or seventh day of the measurement period.

5.3.2 Tn-Clevels

The serum concentrations of Tn-C did not show any clear pattern during the study period. In wound fluid, the concentration of Tn-C was measurable on the first postoperative day and increased from the fifth postoperative day onwards.

6 Discussion

6.1 Methodological considerations

Measurements of the impact of surgery *per se* on the different components of the acute inflammatory response are problematic because such factors as the patient's sex, age, general health and medication, the stage of a malignant disease, the extent of operation, etc. can only be properly controlled in experimental setups, not in clinical surgery.

Also, it is very difficult to differentiate between the effects of anaesthesia and surgery. However, a recent study addressing the effects of one-hour anaesthesia without surgery on phagocytes showed that neither general anaesthesia nor lumbar epidural anaesthesia affected the antibody-dependent cytotoxicity of neutrophils or monocytes or the phagocytic activity of neutrophils (Procopio *et al.* 2001).

The method of collecting samples by silicone rubber tubing has been used in our department for years (Haukipuro *et al.* 1991), and there have been no reported wound healing complications associated with the method. Wound fluid is believed to reflect the wound environment during the healing process (Witte & Barbul 1997).

However, the sample collection and handling were labour-intensive and time-consuming. The schedules were also strict due to the fact that neutrophils had to be examined immediately after sampling. Because of the protocol, only one patient per week could be included. Thus, the number of patients enrolled was restricted and the sample size was relatively small.

The testing of neutrophil chemotaxis, phagocytosis and killing has been a widely used method in our microbiological laboratory, and the technique has been standardized. According to the literature, however, one neutrophil can kill approximately 40-50 bacteria (Clawson & Repine 1976), and the challenge with a bacteria-neutrophil ratio of 5:1 in the phagocytosis test and 1:1 in the killing test in the first trial was presumably easily met.

One difficulty in tests on neutrophil adhesion molecules is that sample handling may affect the expression of CD11b and CD62L (Repo *et al.* 1993, Stibenz & Bührer 1994).

In the first trial, randomization of the patients with the closed envelope method and blinding of the study drug succeeded well, but the organization of the postoperative rounds by keeping the participants blinded to the total white cell counts was sometimes tricky.

6.2 Filgrastim

There were no adverse events that could have been attributed to the treatment with filgrastim. According to the literature, the predominant side effect of filgrastim has been medullary bone pain in 15% to 39% of patients receiving a dose equivalent to ours (Crawford *et al.* 1991, Pettengell *et al.* 1992, Trillet-Lenoir *et al.* 1993, American Society of Clinical Oncology 1994, Maher *et al.* 1994). The obvious reason for the absence of this side effect in the present study was the postoperative pain medication.

The increase of serum alkaline phophatase is a known effect due to release of neutrophil alkaline phosphatase. The phenomenon is harmless and subsides when the medication is discontinued. (Fukumasu *et al.* 1998.)

The healing of laparotomy wounds did not differ between the study groups. The classic study with antineutrophil serum showed that, in the abscence of infection, neutrophils are not essential to wound healing (Simpson & Ross 1972), and in view of the fact that there was only one wound infection in the current study, the result was as expected.

The increase in the total leukocyte and neutrophil counts seen in the present study is a well-documented effect of filgrastim. There was a decrease in neutrophil random migration, chemokinesis and chemotaxis in peripheral blood but not in peritoneal fluid in the filgrastim-treated group compared to the placebo-treated patients. One explanation is that since G-CSF is capable of priming neutrophils (Kitagawa *et al.* 1987), it is possible that the primed neutrophils in the filgrastim-treated patients have migrated into tissues, leaving the less active cell population in blood. Other possible explanations are the loss of responsiveness to a chemotactic response after G-CSF activation or the accelerated production of immature neutrophils with poor chemotactic function.

It is noteworthy that all the differences between placebo and filgrastim, i.e. chemotactic functions, CD11b/CD18 expression and IL-8 concentration, were seen only in peripheral blood but not at the tissue level.

One concern in administering filgrastim in the perioperative period is that leukocytosis can no longer be used as a marker of postoperative infection. However, because the filgrastim treatment did not affect the postoperative temperature or CRP values, this does not seem to be a problem in daily practice.

6.3 Differences between the body compartments

In addition to the effects of filgrastim, the differences between peripheral blood, wound fluid and peritoneal fluid were also examined. No differences in neutrophil functions were seen between the compartments. However, distinct differences in neutrophil adhesion molecules and cytokine concentrations were seen. The results show that local changes in the expression of adhesion molecules may not be reflected systemically, and especially the enormous differences in cytokine concentrations between the different compartments suggest that cytokines are local mediators. In addition to the concentrations, the systemic effects may also be quite different from those occurring at a local site. This must be kept in mind whenever cytokine measurements are used in

clinical trials. It is also important to remember that cytokines work as a network, and analyses of the levels of single cytokines may not reflect the total balance of these mediators (Brennan & Feldmann 2000).

6.4 Effects of surgery on leukocytes

In trials on the effect of surgery on neutrophils (Table 4), the heterogeneity of patient populations, sampling schedules and research methods may lead to partly contradictory results. This makes it very difficult to draw definite conclusions on the topic, and the results of each study must be interpreted in the framework of the methodology used.

The results of our second trial represent the situation in elective colorectal surgery. However, a similar decrease in neutrophil phagocytosis after abdominal surgery has been reported recently (Sietses *et al.* 2000). This decrease may not be important in clinical practice with otherwise healthy patients, but may turn out to be significant in patients with leukopenia or compromised immune functions.

6.5 Effects of surgery on cytokines

Serum G-CSF levels increase in the immediate postoperative period in patients undergoing gastrointestinal surgery, and the rise is proportionate to the degree of surgical stress (Yokota *et al.* 1995). A correlation between the postoperative rises of G-CSF, IL-6 and IL-8 levels has been shown in abdominal surgery (Kato *et al.* 1997) and another between G-CSF and IL-6 levels after thoracoabdominal esophagus surgery (Toda *et al.* 1995). This is in concert with our findings. Elevated postoperative G-CSF levels have also been reported after coronary bypass surgery (Usui *et al.* 1997, Iwasaka *et al.* 2001). These results indicate that G-CSF plays an important role in mediating neutrophilia and possibly also neutrophil activation postoperatively. In the second trial, the cytokine response showed marked interindividual variation, which may impair their use as inflammatory markers in clinical practice.

6.6 Postoperative procollagen propeptides and Tn-C

Our results concerning collagen synthesis are in accordance with the previous reports showing a marked increase of the local procollagen propeptide levels in wound fluid a few days after laparotomy, and the serum values are also accordant with the previously shown pattern (Haukipuro *et al.* 1990, 1991). The actual amounts of PINP and PIIINP in the wound were manyfold compared to the amount of Tn-C. This may reflect the different tasks of these extracellular matrix proteins.

According to our results, Tn-C was detectable in wound fluid from the first postoperative day onwards, indicating a rapid start of the synthesis. In a work using immunohistochemistry, Tn-C was visualized in the wound area approximately two days after wounding (Betz *et al.* 1993). The difference may reflect the different methods used.

Recently, Tn-C serum levels have been shown to reflect disease activity of both inflammatory bowel disease (Riedl *et al.* 2001) and myocarditis (Imanaka-Yoshida *et al.* 2002). The serum levels of Tn-C in our study did not show any clear pattern during the immediate postoperative period and cannot, according to our results, be used as an inflammatory marker postoperatively. Finally, our studies showed no difference in the Tn-C levels between patients with benign or malignant diseases in contrast to a previous study suggesting Tn-C as a possible tumour marker (Schenk *et al.* 1995).

6.7 Future

Due to the local nature of several mediators of inflammation, the concept of taking study samples locally in addition to the traditional blood samples should be considered more often.

Especially for the growing number of patients with compromised host defence mechanisms, infections remain a serious problem. In addition to the traditional methods of preventing surgical infection, such as meticulous surgical technique and prophylactic antibiotics, many new methods will need to be evaluated for clinical use. Immunomodulatory therapies, such as cytokines and their antagonists, may be among them.

In recent studies, the clinical value of filgrastim in infectious diseases has been somewhat dissappointing. The possibility to use filgrastim for infection prophylaxis in surgery may have some theoretical justification, but more clinical studies are needed. Filgrastim treatment is much more expensive than antibiotics, for example, and economic factors, such as cost effectiveness, must also be taken into account.

7 Conclusions

1. Perioperative filgrastim (rhG-CSF) leads to marked neutrophilia in patients undergoing colorectal surgery. Filgrastim reduces neutrophil random migration, chemokinesis and chemotaxis in peripheral blood. Filgrastim does not affect neutrophil phagocytosis or killing capacity in these patients. Filgrastim does not have marked side effects in this patient group.
2. Postoperative expression of the neutrophil adhesion molecules CD11b/CD18 and CD62L differs at the local surgical site from that in peripheral blood. Filgrastim increases only blood CD11b/CD18 expression.
3. There is an abundant release of pro- and anti-inflammatory cytokines IL-1β, TNF-α, IL-6, IL-8, TGF-β and IL-10 into the wound and the peritoneal cavity after colorectal surgery. Filgrastim lowers the postoperative concentrations of IL-8 in blood, but does not have any effect on the local levels of the cytokines studied.
4. Colorectal surgery results in increased numbers of neutrophils and monocytes postoperatively. Neutrophil phagocytosis decreases transiently after the operation. A distinct cytokine response with marked interindividual variation of the cytokines IL-6, IL-8, G-CSF is seen postoperatively.
5. The concentration of Tn-C increases postoperatively in wound fluid during the first postoperative week. The Tn-C-concentration in wound fluid is markedly higher than that in serum.

References

Abraham E & Stevens P (1992) Effects of granulocyte colony-stimulating factor in modifying mortality from Pseudomonas aerigunosa pneumonia after hemorrhage. Crit Care Med 20: 1127–1133.

Agalar F, Iskit AB, Agalar C, Hamaloglu E & Guc MO (1998) The effects of G-CSF treatment and starvation on bacterial translocation in hemorrhagic shock. J Surg Res 78: 143–147.

Van Agthoven M, Fokkens WJ, van de Merwe JP, van Bolhuis EM, Uyl-de Groot CA & Busschbach JJV (2001) Quality of life of patients with refractory chronic rhinosinusitis: effects of filgrastim treatment. Am J Rhinol 15: 231–237.

Allen DB, Maguire JJ, Mahdavian M, Wicke C, Marcocci L, Scheuenstuhl H, Chang M, Le AX, Hopf HW & Hunt TK (1997) Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. Arch Surg 132: 991–996.

Arii K, Tanimura H, Iwahashi M, Tsunoda T, Tani M, Noguchi K, Mizobata S, Hotta T, Nakamori M & Yamaue H (2000) Neutrophil functions and cytokine production in patients with gastric cancer. Hepato-Gastroenterology 47: 291–297.

Arnaout MA (1990) Structure and function of the leukocyte adhesion molecules CD11/CD18. Blood 75: 1037–1050.

American Society of Clinical Oncology recommendations for use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. (1994) J Clin Oncol 12: 2471–2508.

Van der Auwera P, Platzer E, Xu Z-X, Schulz R, Feugeas O, Capdeville R & Edwards DJ (2001) Pharmacodynamics and pharmacokinetics of single doses of subcutaneous pegylated human G-CSF mutant (Ro 25-8315) in healthy volunteers: comparison with single and multiple daily doses of filgrastim. Am J Hematol 66: 245–251.

Avalos BR, Gasson JC, Hedvat C, Quan SG, Baldwin GC, Weisbart RH, Williams RE, Golde DW & DiPersio JF (1990) Human granulocyte colony-stimulating factor: biological activities and receptor characterization on hematopoietic cells and small cell lung cancer lines. Blood 75: 851–857.

Azzara A, Carulli G, Rizzuti-Gullaci A, Minnucci S, Capochiani E & Ambrogi F (1996) Motility of rh-CSF-induced neutrophils in patients undergoing chemotherapy: evidence for inhibition detected by image analysis. Br J Haematol 92: 161–168.

Baba M, Hasegawa H, Nakayabu M, Shimizu N, Suzuki S, Kamada N & Tani K (1995) Establishment and characteristics of a gastric cancer cell line (HuGC-OOHIRA) producing high levels of G-CSF, GM-CSF, and IL-6: the presence of autocrine growth control by G-CSF. Am J Hematol 49: 207–215.

Badia JM, Whawell SA, Scott-Coombes DM, Abel PD, Williamson RCN & Thompson JN (1996) Peritoneal and systemic cytokine response to laparotomy. Br J Surg 83: 347–348.

Bagby GC & Heinrich MC (2000) Growth factors, cytokines and the control of hematopoiesis. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE & McGlave P (eds) Hematology. Basic principles and practice. Churchill Livingstone, Philadelphia, p 154–202.

Bagdade JD, Root RK & Bulger RJ (1974) Impaired leukocyte function in patients with poorly controlled diabetes. Diabetes 23: 9–15.

Barry MC, Condron CM, Watson RWG, Redmond HP, El Jack M, Watson RGK & Bouchier Hayes D (1997) Pre-operative neutrophil and monocyte activation state predicts post-operative neutrophil and monocyte function. Eur J Surg 163: 739–745.

Barsig J, Bundschuh DS, Hartung T, Bauhofer A, Sauer A & Wendel A (1996) Control of fecal peritoneal infection in mice by colony-stimulating factors. J Infect Dis 174: 790–799.

Bauhofer A, Lorenz W, Stinner B, Rothmund M, Koller M, Sitter H, Celik I, Farndon JR, Fingerhut A, Hay J-M, Lefering R, Lorijn RHW, Nyström P-O, Schäfer H, Schein M, Solomkin J, Troidl H, Volk H-D, Wittmann DH, Wyatt J & Lucerne Group for Consensus-assisted Development of the Study Protocol on Prevention of Abdominal Sepsis: Example G-CSF (2001) Granulocyte-colony stimulating factor in the prevention of postoperative infectious complications and sub-optimal recovery from operation in patients with colorectal cancer and increased preoperative risk (ASA 3 and 4). Protocol of a controlled clinical trial developed by consensus of an international study group. Part two: design of the study. Inflamm Res 50: 187–205.

Begley CG, Lopez AF, Nicola NA, Warren DJ, Vadas MA, Sanderson CJ & Metcalf D (1986) Purified colony-stimulating factors enhance the survival of human neutrophils and eosinophils in vitro: a rapid and sensitive microassay for colony-stimulating factors. Blood 68: 162–166.

Van Berge Henegouwen MI, van der Poll T, van Deventer SJH & Gouma DJ (1998) Peritoneal cytokine release after elective gastrointestinal surgery and postoperative complications. Am J Surg 175: 311–316.

Bermudez LE, Petrofsky M & Stevens P (1998) Treatment with recombinant granulocyte colony-stimulating factor (Filgrastim™) stimulates neutrophils and tissue macrophages and induces an effective non-specific response against Mycobacterium avium in mice. Immunology 94: 297–303.

Betz P, Nerlich A, Tübel J, Penning R & Eisenmenger W (1993) Localization of tenascin in human skin wounds – an immunohistochemical study. Int J Leg Med 105: 325–328.

Beveridge RA, Miller JA, Kales AN, Binder RA, Robert NJ, Harvey JH, Windsor K, Gore I, Cantrell J, Thompson KA, Taylor WR, Barnes HM, Schiff SA, Shields JA, Cambareri RJ, Butler TP, Meister RJ, Feigert JM, Norgard MJ, Moraes MA, Helvie WW, Patton GA, Mundy LJ, Henry D, Mason B, Staddon A, Ford P, Katcher D, Houck W, Major WB, Gemma NW, Kay G, Priest E, Sowroy P, Bank B, Leibach S, Reisel H, Grad G, Warren RD, Ueno WM, Smith LF, Dobrzynski RF & Sheridan MJ (1998) A comparison of efficacy of sargramostim (yeast-derived RhuGM-CSF) and filgrastim (bacteria-derived RhuG-CSF) in the therapeutic setting of chemotherapy-induced myelosuppression. Cancer Invest 16: 366–373.

Bober LA, Grace MJ, Pugliese-Sivo C, Rojas-Triana A, Waters T, Sullivan LM & Narula SK (1995) The effect of GM-CSF and G-CSF on human neutrophil function. Immunopharmacology 29: 111–119.

Bokoch GM (1995) Chemoattractant signaling and leukocyte activation. Blood 86: 1649–1660.

Boneberg E-M, Hareng L, Gantner F, Wendel A & Hartung T (2000) Human monocytes express functional receptors for granulocyte colony-stimulating factor that mediate suppression of monokines and interferon-gamma. Blood 95: 270–276.

Borleffs JCC, Bosschaert M, Vrehen HM, Schneider MME, van Strijp J, Small MK & Borkett KM (1998) Effect of escalating doses of recombinant human granulocyte colony-stimulating factor (filgrastim) on circulating neutrophils in healthy subjects. Clin Ther 20: 722–736.

Bouchama A, Khan B, Djazmati W & Shukri K (1999) Hematopoietic colony-stimulating factors for neutropenic patients in the ICU. Intensive Care Med 25: 1003–1005.

Bowers TK, O' Flaherty J, Simmons RL & Jacob HS (1977) Postsurgical granulocyte dysfunction: studies in healthy kidney donors. J Lab Clin Med 90: 720–727.

Boxer LA, Hutchinson R & Emerson S (1992) Recombinant human granulocyte-colony-stimulating factor in the treatment of patients with neutropenia. Clin Immun Immunopathol 62: 39–46.

Brandt ML (2001) Secondary immunodeficiency inducted by surgery or trauma. In: Rich RR, Fleisher TA, Kotzin BL & Schroeder HW (eds) Clinical Immunology. Principles and practice. Mosby International Limited, London, p 44.1–44.7.

Brennan FM & Feldmann M (2000) Cytokine networks. In: Balkwill F (ed) The cytokine network. Oxford University Press, Oxford, p 49–70.

Bronchud MH, Howell A, Crowther D, Hopwood P, Souza L & Dexter TM (1989) The use of granulocyte colony-stimulating factor to increase the intensity of treatment with doxorubicin in patients with advanced breast and ovarian cancer. Br J Cancer 60: 121–125.

Burgess AW & Metcalf D (1980) Characterization of a serum factor stimulating the differentiation of myelomonocytic leukemic cells. Int J Cancer 26: 647–654.

Bussolino F, Wang JM, Defilippi P, Turrini F, Sanavio F, Edgell CS, Aglietta M, Arese P & Mantovani A (1989) Granulocyte- and granulocyte-macrophage colony-stimulating factors induce human endothelial cells to migrate and proliferate. Nature 337: 471–473.

Bussolino F, Ziche M, Wang JM, Alessi D, Morbidelli L, Cremona O, Bosia A, Marchisio PC & Mantovani A (1991) In vitro and in vivo activation of endothelial cells by colony-stimulating factors. J Clin Invest 87: 986–995.

Bönig H, Silbermann S, Weller S, Kirschke R, Körholz D, Janssen G, Göbel U & Nürnberger W (2001) Glycosylated vs non-glycosylated granulocyte colony-stimulating factor (G-CSF) -results of a prospective randomised monocentre study. Bone Marrow Transplant 28: 259–264.

Cabié A, Fiting C, Farkas JC, Laurian C, Cormier JM, Carlet J & Cavaillon JM (1992) Influence of surgery on in-vitro production by human monocytes. Cytokine 1992; 4: 576–580.

Cainzos MA (1998) Antibiotic prophylaxis New Horiz 6: 11–19.

Cairo MS, Mauss D, Kommareddy S, Norris K, van de Ven C & Modanlou H (1990) Prophylactic or simultaneous administration of recombinant human granulocyte colony-stimulating factor in the treatment of group B streptococcal sepsis in neonatal rats. Pediatric Res 27: 612–616.

Carlos TM & Harlan JM (1994) Leukocyte-endothelial adhesion molecules. Blood 84: 2068–2101.

Casimir CM & Teahan CG (1994) The respiratory burst of neutrophils and its deficiency. In: Hellewell PG & Williams TJ (eds) Immunopharmacology of neutrophils. Academic Press Limited, London, p 27–54.

Cavaillon J-M & Duff G (1999) Cytokines and the cellular mechanism of inflammation. In: Thèze J (ed) The cytokine network and immune functions. Oxford University Press, Oxford, p 251–261.

Cebon J, Layton JE, Maher D & Morstyn G (1994) Endogenous haemopoietic growth factors in neutropenia and infection. Br J Haematol 86: 265–274.

Chuong C & Chen H (1991) Enhanced expression of neural cell adhesion molecules and tenascin (cytotactin) during wound healing. Am J Pathol 138: 427–440.

Claesson BEB & Holmlund DEW (1988) Predictors of intraoperative bacterial contamination and postoperative infection in elective colorectal surgery. J Hosp Infect 11: 127–135.

Clark RAF (1996) Wound repair. Overview and general considerations. In: Clark RAF (ed) The molecular and cellular biology of wound repair. Plenum Press, New York, p 3–50.

Clawson CC & Repine JE (1976) Quantitation of maximal bactericidal capacity in human neutrophils. J Lab Clin Med 88: 316–327.

Çolak T, Îpek T, Paksoy M, Polat E, Uygun N & Kayabasi B (2001) The effects of cefephim, G-CSF, and sucralfate on bacterial translocation in experimentally induced acute pancreatitis. Surg Today 31: 502–506.

Crawford J, Ozer H, Stoller R, Johnson D, Lyman G, Tabbara I, Kris M, Grous J, Picozzi V, Rausch G, Smith R, Gradishar W, Yahanda A, Vincent M, Stewart M & Glaspy J (1991) Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer. N Engl J Med 325: 164–170.

Cruse PJE & Foord R (1980) The epidemiology of wound infection. A 10-year prospective study of 62, 939 wounds. Surg Clin North Am 60: 27–40.

Cullen BF, Hume RB & Chretien PB (1975) Phagocytosis during general anesthesia in man. Anesth Analg 54: 501–504.

Dale DC, Bonilla MA, Davis MW, Nakanishi AM, Hammond WP, Kurzberg J, Wang W, Jakubowski A, Winton E, Lalezari P, Robinson W, Glaspy JA, Emerson S, Gabrilove J, Vincent M & Boxer LA (1993) A randomized controlled phase III trial of recombinant human granulocyte colony-stimulating factor (Filgrastim) for treatment of severe chronic neutropenia. Blood 81: 2496–2502.

Dale DC (1995) Where now for colony-stimulating factors? Lancet 346: 135–136.

Daschner FD, Grundmann H, Anding K & Lemmen S (1995) Combined effect of human neutrophils, ceftazidime and granulocyte colony-stimulating factor on killing of Escherichia coli. Eur J Clin Microbiol Infect Dis 14: 536–539.

Davies JM, Sheppard K & Fletcher J (1983) The effect of surgery on the activity of neutrophil granule proteins. Br J Haematol 53: 5–13.

Davies M & Hagen P (1997) Systemic inflammatory response syndrome. Br J Surg 84: 920–935.

Delves PJ & Roitt IM (2000) The immune system. First of two parts. N Engl J Med 343: 37–49.

Demetri GD & Griffin JD (1991) Granulocyte colony-stimulating factor and its receptor. Blood 78: 2791–2808.

Demetri GD (1994) The use of hematopoietic growth factors to support cytotoxic chemotherapy for patients with breast cancer. Hematol Oncol Clin N Am 8: 233–249.

Demling RH, Lalonde C & Ikegami K (1994) Physiologic support of the septic patient. In: Deitch E (ed) Surgical infections. Surg Clin N Am 74: 637–658.

Van Dijk WC, Verbrugh HA, van Rijswijk REN, Vos A & Verhoef J (1982) Neutrophil function, serum opsonic activity, and delayed hypersensitivity in surgical patients. Surgery 92: 21–29.

Dinarello CA (1997) Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. Chest 112: 321–329.

Doherty NS & Janusz MJ (1994) Neutrophil proteases: their physiological and pathological roles. In: Hellewell PG & Williams TJ (eds) Immunopharmacology of neutrophils. Academic Press Limited, London, p 55–94.

Dunne JR, Dunkin BJ, Nelson S & White JC (1996) Effects of granulocyte colony-stimulating factor in a nonneutropenic rodent model of Escherichia coli peritonitis. J Surg Res 61: 348–354.

Eaves-Pyles T & Alexander JW (1996) Granulocyte colony-stimulating factor enhances killing of translocated bacteria but does not affect barrier function in a burn mouse model. J Trauma 41: 1013–1017.

Eckes B, Aumailley M & Krieg T (1996) Collagens and the reestablishment of dermal integrity. In: Clark RAF (ed) The molecular and cellular biology of wound repair. Plenum Press, New York, p 493–512.

Eissner G, Lindner H, Reisbach G, Klauke I & Holler E (1997) Differential modulation of IL-1-induced endothelial adhesion molecules and transendothelial migration of granulocytes by G-CSF. Br J Haematol 97: 726–733.

El-Maallem H & Fletcher J (1981) Effects of surgery on neutrophil granulocyte function. Infect Immun 32: 38–41.

Endler M, Endler TA & Zielinski C (1982) Influence of hip arthroplasty upon chemotactic behaviour of leucocytes. Acta Orthop Scand 53: 795–798.

Erickson HP & Lightner VA (1988) Hexabrachion protein (tenascin, cytotactin, brachionectin) in connective tissues, embryonic brain, and tumors. Adv Cell Biol 2: 55–90.

Esparza B, Sánchez H, Ruiz M, Barranquero M, Sabino E & Merino F (1996) Neutrophil function in elderly persons assessed by flow cytometry. Immunol Invest 25: 185–190.

Fabian I, Kletter Y, Bleiberg I, Gadish M, Naparsteck E & Slavin S (1991) Effect of exogenous recombinant human granulocyte and granulocyte-macrophage colony-stimulating factor on neutrophil function following allogeneic bone marrow transplantation. Exp Hematol 19:868–873.

Fanning J, Colgrove M & Phibbs G (2000) Cisplatin-paclitaxel-cyclophosphamide with G-CSF in primary advanced epithelial ovarian cancer. Gyn Oncol 79: 97–100.

Fink MP, O'Sullivan BP, Menconi MJ, Wollert SP, Wang H, Youssef ME & Bellelsle JM (1993) Effect of granulocyte colony-stimulating factor on systemic and pulmonary responses to endotoxin in pigs. J Trauma 34: 571–577.

Foëx BA & Shelly MP (1996) The cytokine response to critical illness. J Accid Emerg Med 13: 154–162.

Frampton JE, Lee CR & Faulds D (1994) Filgrastim. A review of its pharmacological properties and therapeutic efficacy in neutropenia. Drugs 48: 731–760.

Fukumasu H, Fukumasu Y & Ogita S (1998) Elevation in plasma alkaline phosphatase level during rhG-CSF administration. Granulocytopenic patients with gynecologic cancers treated with cancer chemotherapy.

Fukunaga R, Ishizaka-Ikeda E, Seto Y & Nagata S (1990) Expression cloning of a receptor for murine granulocyte colony-stimulating factor. Cell 61: 341–350.

Furumkin LR (1997) Role of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the treatment of patients with HIV infection. Curr Opin Hematol 4: 200–206.

Gabay C & Kushner I (1999) Acute phase proteins and other systemic responses to inflammation. N Engl J Med 340: 448–454.

Gabrilove JL, Jakubowski A, Fain K, Grous J, Scher H, Sternberg C, Yagoda A, Clarkson B, Bonilla MA, Oettgen HF, Alton K, Boone T, Altrock B, Welte K & Souza L (1988) Phase I study of granulocyte colony-stimulating factor in patients with transitional cell carcinoma of the urothelium. J Clin Invest 82: 1454–1461.

Gabrilove JL (1991) Colony-stimulating factors: clinical status. In: DeVita VT, Hellman S & Rosenberg SA (eds) Biologic therapy of cancer. J. B. Lippincott Company. Philadelphia, p 445–463.

Gahmberg CG (1997) Leukocyte adhesion: CD11/CD18 integrins and intercellular adhesion molecules. Curr Opin Cell Biol 9: 643–650.

Gamelli RL, He L-K & Liu H (1995) Recombinant human granulocyte colony-stimulating factor treatment improves macrophage suppression of granulocyte and macrophage growth after burn and burn wound infection. J Trauma 39: 1141–1147.

Ganz T (1993) Macrophage function. New Horiz 1: 23–27.

Glaspy JA, Baldwin GC, Robertson PA, Souza L, Vincent M, Ambersley J & Golde DW (1988) Therapy for neutropenia in hairy cell leukemia with recombinant human granulocyte colony-stimulating factor. Ann Int Med 109: 789–795.

Goldsby RA, Kindt TJ & Osborne BA (2001) Leukocyte migration and inflammation. In Goldsby RA, Kindt TJ & Osborne BA (eds) Immunology. WH Freeman and Company, New York, p 371–393.

Gordon MY (1994) Origin and development of neutrophils. In: Hellewell PG & Williams TJ (eds) Immunopharmacology of neutrophils. Academic Press Limited, London, p 1–26.

Gough A, Clapperton M, Rolando N, Foster AVM, Philpott-Howard J & Edmonds ME (1997) Randomised placebo-controlled trial of granulocyte colony-stimulating factor in diabetic foot infection. Lancet 350: 855–859.

Granger DN & Kubes P (1994) The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesions. J Leukoc Biol 55: 662–675.

Greif R, Akça O, Horn E-P, Kurz A & Sessler DI (2000) Supplemental perioperative oxygen to reduce the incidence of surgical wound infection. N Engl J Med 342: 161–167.

Gross-Weege W, Weiss M, Schneider M, Wenning M, Harms B, Dumon K, Ohmann C & Röher H-D (1997a) Safety of a low-dosage Filgrastim (rhG-CSF) treatment in non-neutropenic surgical intensive care patients with an inflammatory process. Intensive Care Med 23: 16–22.

Gross-Weege W, Dumon K, Dahmen A, Schneider EM & Röher H-D (1997b) Granulocyte colony-stimulating factor (G-CSF) serum levels in surgical intensive care patients. Infection 25: 213–216.

Gruson D, Hilbert G, Vargas F, Valentino R, Chene G, Boiron J-M, Reiffers J, Gbikpi-Benissan G & Cardinaud J-P (2000) Impact of colony-stimulating factor therapy on clinical outcome and frequency rate of nosocomial infections in intensive care unit neutropenic patients. Crit Care Med 28: 3155–3160.

Guillou PJ (1995) Adjuvant biological response modifiers after major surgery or trauma. Br J Surg 82: 721–723.

Gupta S (1987) Immune response following surgical trauma. Crit Care Clin 3: 405–415.

Görgen I, Hartung T, Leist M, Niehörster M, Tiegs G, Uhlig S, Weitzel F & Wendel A (1992) Granulocyte colony-stimulating factor treatment protects rodents against lipopolysaccharide-induced toxicity via suppression of systemic tumor necrosis factor-α. J Immunol 149: 918–924.

De Haas M, Kerst JM, van der Schoot CE, Calafat J, Hack CE, Nuijens JH, Roos D, van Oers RHJ & von dem Borne AEGKr (1994) Granulocyte colony-stimulating factor administration to healthy volunteers: analysis of the immediate activating effects on circulating neutrophils. Blood 84: 3885–3894.

Haley RW, Culver DH, Morgan WM, White JW, Emori TG & Hooton TM (1985) Identifying patients at high risk of surgical wound infection. Am J Epidemiol 121: 206–215.

Hammond WB, Price TH, Souza LM & Dale DC (1989) Treatment of cyclic neutropenia with granulocyte colony-stimulating factor. N Engl J Med 320: 1306–1311.

Hartung T, Döcke W-D, Gantner F, Krieger G, Sauer A, Stevens P, Volk H-D & Wendel A (1995) Effect of granulocyte colony-stimulating factor treatment on ex vivo blood cytokine response in human volunteers. Blood 85: 2482–2489.

Hartung T (1998) Anti-inflammatory effects of granulocyte colony-stimulating factor. Curr Opin Hematol 5: 221–225.

Hartung T (1999) Anti-inflammatory aspects of filgrastim and impact on IL-2 release. J Hematother Stem Cell Res 8: 21–22.

Hartung T, Doecke W-D, Bundschuh D, Foote MA, Gantner F, Hermann C, Lenz A, Milwee S, Rich B, Simon B, Volk H-D, von Aulock S & Wendel A (1999) Effect of filgrastim treatment on inflammatory cytokines and lymphocyte functions. Clin Pharmacol Ther 66: 415–424.

Haslett C, Savill JS & Meagher L (1989) The neutrophil. Curr Opin Immunol 2: 10–18.

Haslett C & Henson P (1996) Resolution of inflammation. In: Clark RAF (ed) The molecular and cellular biology of wound repair. Plenum Press, New York, p 143–168.

Haukipuro K, Risteli L, Kairaluoma MI & Risteli J (1990) Aminoterminal propeptide of type III procollagen in serum during wound healing in human beings. Surgery 107: 381–388.

Haukipuro K, Melkko J, Risteli L, Kairaluoma MI & Risteli J (1991) Synthesis of type I collagen in healing wounds in humans. Ann Surg 213: 75–80.

Haukipuro K, Melkko J, Risteli L, Kairaluoma MI & Risteli J (1992) Connective tissue response to major surgery and postoperative infection. Eur J Clin Invest 22: 333–340.

Heard SO, Fink MP, Gamelli RL, Solomkin JS, Joshi M, Trask AL, Fabian TC, Hudson LD, Gerold KB, Logan ED & The Filgrastim study group (1998) Effect of prophylactic administration of recombinant human granulocyte colony-stimulating factor (filgrastim) on the frequency of nosocomial infections in patients with acute traumatic brain injury or cerebral hemorrhage. Crit Care Med 26: 748–754.

Hebert JC, O'Reilly M & Gamelli RL (1990) Protective effect of recombinant human granulocyte colony-stimulating factor against pneumococcal infections in splenectomized mice. Arch Surg 125: 1075–1078.

Hisano S, Sakamoto K, Ishiko T, Kamohara H & Ogawa M (1997) IL-6 and soluble IL-6 receptor levels change differently after surgery both in the blood and in the operative field. Cytokine 9: 447–452.

Holzheimer RG & Steinmetz W-G (2000) Local and systemic concentrations of pro- and anti-inflammatory cytokines in human wounds. Eur J Med Res 5: 347–355.

Hommes DW, Meenan J, Dijkhuizen S, Ten Kate FJW, Tytgat GNJ & van Deventer SJH (1996) Efficacy of recombinant granulocyte colony-stimulating factor (rhG-CSF) in experimental colitis. Clin Exp Immunol 106: 529–533.

Hopf HW, Hunt TK, West JM, Blomquist P, Goodson WH, Jensen JA, Jonsson K, Paty PB, Rabkin JM, Upton RA, von Smitten K & Whitney JD (1997) Wound tissue oxygen tension predicts the risk of wound infection in surgical patients. Arch Surg 132: 997–1004.

Hu B & Yasui K (1997) Effects of colony-stimulating factors (CSFs) on neutrophil apoptosis: possible roles at inflammation site. Int J Hematol 66: 179–188.

Hübel K, Mansmann G, Schäfer H, Oberhäuser F, Diehl V & Engert A (2000) Increase of anti-inflammatory cytokines in patients with esophageal cancer after perioperative treatment with G-CSF. Cytokine 12: 1797–1800.

Høgevold HE, Lyberg T, Kähler H & Reikerås O (1996) Expression of beta-2-integrins and L-selectin by leukocytes and changes in acute-phase reactants in total hip replacement surgery. Eur Surg Res 28: 190–200.

Ichiishi E, Yoshikawa T, Kogawa T, Yoshida N & Kondo M (2000) Possible paracrine growth of adenocarcinoma of the stomach induced by granulocyte colony-stimulating factor produced by squamous cell carcinoma of the oesophagus. Gut 46: 432–434.

Inoue M, Kato H, Mukai S, Kawahito Y, Asai K, Kimura S, Yamamura Y, Sano H, Sugino S & Kondo M (1994) Recombinant human granulocyte colony-stimulating factor augments cytotoxicity of OK-432-induced polymorphonuclear leukocytes. Int J Immunopharmac 16: 19–28.

Ishikawa K, Tanaka H, Matsuoka T, Shimazu T, Yoshioka T & Sugimoto H (1998) Recombinant human granulocyte colony-stimulating factor attenuates inflammatory responses in septic patients with neutropenia. J Trauma 44: 1047–1055.

Iwasaka H, Kitano T, Miyakawa H, Unoshima M, Shinguu C, Matsumoto S & Noguchi T (2001) Neutrophilia and granulocyte colony-stimulating factor levels after cardiopulmonary bypass. Can J Anesth 48: 81–84.

Jimenez MF, Watson RGW, Parodo J, Evans D, Foster D, Steinberg M, Rotstein OD & Marshall JC (1997) Dysregulated expression of neutrophil apoptosis in the systemic inflammatory response syndrome. Arch Surg 132: 1263–1270.

Jensen RH, Storgaard M, Vedelsdal R & Obel N (1995) Impaired neutrophil chemotaxis after cardiac surgery. Scand J Thor Cardiovasc Surg 29: 115–118.

Juhasz I, Murphy GF, Yan H, Herlyn M & Albelda SM (1993) Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous wound healing in vivo. Am J Pathol 143: 1458–1469.

Kaarteenaho-Wiik R, Tani T, Sormunen R, Soini Y, Virtanen I & Pääkkö P (1996) Tenascin immunoreactivity as a prognostic marker in usual interstitial pneumonia. Am J Respir Crit Care Med 154: 511–518.

Kaarteenaho-Wiik R, Kinnula V, Herva R, Pääkkö P, Pöllänen R & Soini Y (2001) Distribution and mRNA expression of tenascin-C in developing human lung. Am J Respir Cell Mol Biol 25: 341–6.

Kanazawa M, Ishizaka A, Hasegawa N, Suzuki Y & Yokoyama T (1992) Granulocyte colony-stimulating factor does not enhance endotoxin-induced acute lung injury in guinea pigs. Am Rev Respir Dis 145: 1030–1035.

Kato M, Suzuki H, Murakami M, Akama M, Matsukawa S & Hashimoto Y (1997) Elevated plasma levels of interleukin-6, interleukin-8, and granulocyte colony-stimulating factor during and after major abdominal surgery. J Clin Anesth 9: 293–298.

Kaushansky K, Lin N & Adamson JW (1988) Interleukin 1 stimulates fibroblasts to synthesize granulocyte-macrophage and granulocyte colony-stimulating factors. Mechanism for the hematopoietic response to inflammation. J Clin Invest 81: 92–97.

Kawakami M, Tsutsumi H, Kumakawa T, Abe H, Hirai M, Kurosawa S, Mori M & Fukushima M (1990) Levels of serum granulocyte colony-stimulating factor in patients with infections. Blood 76: 1962–1964.

Kerst JM, de Haas M, van der Schoot CE, Slaper-Cortenbach ICM, Kleijer M, von dem Borne AEGK & van Oers RHJ (1993) Recombinant granulocyte colony-stimulating factor administration to healthy volunteers: induction of immunophenotypically and functionally altered neutrophils via an effect on myeloid progenitor cells. Blood 82: 3265–3272.

Khan FA, Kamal RS, Mithani CH & Khurshid M (1995) Effect of general anaesthesia and surgery on neutrophil function. Anaesthesia 50: 769–775.

Kitagawa S, Yuo A, Souza LM, Saito M, Miura Y & Takaku F (1987) Recombinant human granulocyte colony-stimulating factor enhances superoxide release in human granulocytes stimulated by the chemotactic peptide. Biochem Biophys Res Commun 14: 1143–1146.

Klava A, Windsor ACJ, Ramsden CW & Guillou PJ (1997) Enhanced polymorphonuclear leukocyte adhesion after surgical injury. Eur J Surg 163: 747–752.

Koeffler HP, Gasson J, Ranyard J, Souza L, Shepard M & Munker R (1987) Recombinant human TNFα stimulates production of granulocyte colony-stimulating factor. Blood 70: 55–59.

Koizumi T, Kubo K, Shinozaki S, Koyama S, Kobayashi T & Sekiguchi M (1993) Granulocyte colony-stimulating factor does not exacerbate endotoxin-induced lung injury in sheep. Am Rev Respir Dis 148: 132–137.

Kojima S, Fukuda M, Miyajima Y, Matsuyama T & Horibe K (1991) Treatment of aplastic anemia in children with recombinant human granulocyte colony-stimulating factor. Blood 77: 937–941.

Koukoulis GK, Gould VE, Bhattacharyya A, Gould JE, Howeedy AA & Virtanen I (1991) Tenascin in normal, reactive, hyperplastic and neoplastic tissues: biologic and pathologic implications. Hum Pathol 22: 636–643.

Kragsbjerg P, Jones I, Vikerfors T & Holmberg H (1995) Diagnostic value of blood cytokine concentrations in acute pneumonia. Thorax 50: 1253–1257.

Krausz MM, Hartzstark Z, Shlomai Z, Gross D, Matzner Y, Eldor A, Vlodavsky I & Ben Bassat H (1988) Decreased neutrophil thromboxane A_2 and endothelial PGI_2 production in the postoperative period. Ann Surg 208: 78–84.

Krohn CD, Reikerås O & Aasen AO (1999) The cytokines IL-1β, and IL-1 receptor antagonist, IL-2 and IL-2 soluble receptor-α, IL-6 and IL-6 soluble receptor, TNF-α and TNF soluble receptor I, and IL 10 in drained and systemic blood after major orthopaedic surgery. Eur J Surg 165: 101–109.

Kropec A, Lemmen SW, Grundmann HJ, Engels I & Daschner FD (1995) Synergy of simultaneous administration of ofloxacin and granulocyte colony-stimulating factor in killing of Escherichia coli by human neutrophils. Infection 23: 298–300.

Kuhns DB, Long Priel DA & Gallin JI (1995a) Loss of L-selectin (CD62L) on human neutrophils following exudation in vivo. Cell Immunol 164: 306–310.

Kuhns DB, Alvord WG & Gallin JI (1995b) Increased circulating cytokines, cytokine-antagonists, and E-selectin after intravenous administration of endotoxins in humans. J Infect Dis 171: 145–152.

Kuritzkes DR (2000) Neutropenia, neutrophil dysfunction, and bacterial infection in patients with human immunodeficiency virus disease: the role of granulocyte colony-stimulating factor. Clin Infect Dis 30: 256–260.

Kurz A, Sessler DI & Lenhardt R (1996) Perioperative normothermia to reduce the incidence of surgical wound infection and shorten hospitalization. N Engl J med 334: 1209–1215.

Lang CH, Bagby GJ, Dobrescu C, Nelson S & Spitzer JJ (1992) Effect of granulocyte colony-stimulating factor on sepsis-induced changes in neutrophil accumulation and organ glucose uptake. J Infect Dis 166: 336–343.

Lasky LA (1992) Selectins: interpreters of cell-specific carbohydrate information during inflammation. Science 258: 964–969.

Latijnhouwers MA, Bergers M, van Bergen BH, Spruijt KI, Andriessen MP & Schalkwijk J (1996) Tenascin expression during wound healing in human skin. J Pathol 178: 30–35.

Lawrence MB & Springer TA (1991) leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. Cell 65: 859–873.

Layton JE, Hall NE, Connell F, Venhorst J & Treutlein HR (2001) Identification of ligand-binding site III on the immunoglobulin-like domain of the granulocyte colony-stimulating factor receptor. J Biol Chem 276: 36779–36787.

Lee RC & Doong H (1993) Control of matrix production during tissue repair. In: Andersen D (ed) Advances in wound healing and tissue repair. Master Series in Surgery. World Medical Press, New York, p 1–25.

Lieschke GJ & Burgess AW (1992) Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. N Engl J Med 327: 28–35.

Lieschke GJ, Grail D, Hodgson G, Metcalf D, Stanley E, Cheers C, Fowler KJ, Basu S, Zhan YF & Dunn AR (1994) Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. Blood 84: 1737–1746.

Lightner VA, Gumkowski F, Bigner DD & Erickson HP (1989) Tenascin/hexabrachion in human skin: biochemical identification and localization by light and electron microscopy. J Cell Biol 108: 2483–2493.

Lightner VA (1994) Tenascin: does it play a role in epidermal morphogenesis and homeostasis? J Invest Dermatol 102: 273–277.

Liles WC, Huang JE, van Burik J-AH, Bowden RA & Dale DC (1997) Granulocyte colony-stimulating factor administered in vivo augments neutrophil-mediated activity against opportunistic fungal pathogens. J Infect Dis 175: 1012–1015.

Lin E, Calvano SE & Lowry SF (1998) Cytokine response in abdominal surgery. In: Schein M & Wise L (eds) Cytokines and the abdominal surgeon. RG Landes Company, Austin, p 17–34.

Lindemann A, Herrmann F, Oster W, Haffner G, Meyenburg W, Souza LM & Mertelsmann (1989) Hematologic effects of recombinant human granulocyte colony-stimulating factor in patients with malignancy. Blood 74: 2644–2651.

Van Lindert ACM, Symons EA, Damen BFM & Heintz APM (1995) Wound healing after radical vulvectomy and inguino-femoral lymphadenectomy: experience with granulocyte colony stimulating factor (filgrastim, r-methuG-CSF. Eur J Obstr Gyn 62: 217–219.

Lloyd AR, Biragyn A, Johnston JA, Taub DD, Xu L, Michiel D, Sprenger H, Oppenheim JJ & Kelvin DJ (1995) Granulocyte colony-stimulating factor and lipopolysaccharide regulate the expression of interleukin 8 receptors on polymorphonuclear leukocytes. J Biol Chem 270: 28188–28192.

Lord BI, Woolford LB & Molineux G (2001) Kinetics of neutrophil production in normal and neutropenic animals during the response to filgrastim (r-metHu G-CSF or filgrastim SD/01 (PEG-r-metHu G-CSF). Clin Cancer Res 7: 2085–2090.

Lorenz W, Reimund K-P, Weitzel F, Celik I, Kurnatowski M, Schneider C, Mannheim W, Heiske A, Neumann K, Sitter H & Rothmund M (1994) Granulocyte colony-stimulating factor prophylaxis before operation protects against lethal consequences of postoperative peritonitis. Surgery 116: 925–934.

Lorenz W, Stinner B, Bauhofer A, Rothmund M, Celik I, Fingerhut A, Koller M, Lorijn RHW, Nyström PO, Sitter H, Schein M, Solomkin JS, Troidl H, Wyatt J, Wittmann DH & Lucerne Group for Consensus-assisted Development of the Study Protocol on Prevention of Abdominal Sepsis: Example G-CSF (2001) Granulocyte-colony stimulating factor in the prevention of postoperative infectious complications and sub-optimal recovery from operation in patients with colorectal cancer and increased preoperative risk (ASA 3 and 4). Protocol of a controlled clinical trial developed by consensus of an international study group. Part one: rationale and hypothesis. Inflamm Res 50: 115–122.

Lundblad R, Nesland JM & Giercksky K (1996) Granulocyte colony-stimulating factor improves survival rate and reduces concentrations of bacteria, endotoxin, tumor necrosis factor, and endothelin-1 in fulminant intra-abdominal sepsis in rats.Crit Care Med 24: 820–826.

Luomanen M & Virtanen I (1993) Distribution of tenascin in healing incision, excision and laser wounds. J Oral Pathol Med 22: 41–45.

Luster AD (1998) Chemokines – chemotactic cytokines that mediate inflammation. N Engl J Med 338: 436–445.

Mackie EJ, Halfter W & Liverani D (1988) Induction of tenascin in healing wounds. J Cell Biol 107: 2757–2767.

Maher DW, Lieschke GJ, Green M, Bishop J, Stuart-Harris R, Wolf M, Sheridan WP, Kefford RF, Cebon J, Olver I, McKendrick J, Toner G, Bradstock K, Lieschke M, Cruickshank S, Tomita DK, Hoffman EW, Fox RM & Morstyn G (1994) Filgrastim in patients with chemotherapy-induced febrile neutropenia. A double-blind, placebo-controlled trial. Ann Intern Med 121: 492–501.

Mamounas EP, Anderson S, Wickerham DL, Clark R, Stoller R, Hamm JT, Stewart JA, Bear HD, Glass AG, Bornstein D & Fisher B (1994) The efficacy of recombinant human granulocyte colony-stimulating factor and recombinant human granulocyte macrophage colony-stimulating factor in permitting the administration of higher doses of cyclophosphamide in a doxorubicin-cyclophosphamide combination. Am J Clin Oncol 17: 374–381.

Martin TR (1997) Overview of cytokine networks in lung injury. In: Pratter MR & Nelson S (eds) Cytokines and pulmonary infection. Part II: the role of cytokines in systemic and pulmonary medicine. American Lung Association, p 19–28.

Mealy K, O'Farrelly C, Stephens R & Feighery C (1987) Impaired neutrophil function during anesthesia and surgery is due to serum factors. J Surg Res 43: 393–397.

Melling AC, Ali B, Scott EM & Leaper DJ (2001) Effects of preoperative warming on the incidence of wound infection after clean surgery: a randomised controlled trial. Lancet 358: 876–880.

Metcalf D (1980) Clonal extinction of myelomonocytic leukemic cells by serum from mice injected with endotoxin. Int J Cancer 25: 225–233.

Metcalf D (1989) The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. Nature 339: 27–30.

Mooney DP, Gamelli RL, O'Reilly M & Hebert JC (1988) Recombinant human granulocyte colony-stimulating factor and Pseudomonas burn wound sepsis. Arch Surg 123: 1353–1357.

Morley JI & Kushner I (1982) Serum C-reactive protein levels in disease. Ann N Y Acad Sci 389: 406–418.

Morstyn G, Souza LM, Keech J, Sheridan W, Campbell L, Alton NK, Green M & Metcalf D (1988) Effect of granulocyte colony stimulating factor on neutropenia induced by cytotoxic chemotherapy. Lancet 1: 667–671.

Morstyn G, Campbell L, Lieschke G, Layton JE, Maher D, O'Connor M, Green M, Sheridan W, Vincent M, Alton K, Souza L, McGrath K & Fox RM (1989) Treatment of chemotherapy-induced neutropenia by subcutaneously administered granulocyte colony-stimulating factor with optimization of dose and duration of therapy. J Clin Oncol 7: 1554–1562.

Moudgil GC, Pandya AR & Ludlow DJ (1981) Influence of anaesthesia and surgery on neutrophil chemotaxis. Canad Anaesth Soc J 28: 232–238.

Murakami R, Yamaoka I & Sakakura T (1989) Appearance of tenascin in healing skin of the mouse: possible involvement in seaming of wounded tissue. Int J Dev Biol 33: 439–444.

Nagata S & Fukunaga R (1991) Granulocyte colony-stimulating factor and its receptor. Prog Growth Factor Res 3: 131–141.

Negrin RS, Haeuber DH, Nagler A, Kobayashi Y, Sklar J, Donlon T, Vincent M & Greenberg PL (1990) Maintenance treatment of patients with myelodysplastic syndromes using recombinant human granulocyte colony-stimulating factor. Blood 76: 36–43.

Nelson S, Summer W, Bagby G, Nakamura C, Stewart L, Lipscomb G & Andresen J (1991) Granulocyte colony-stimulating factor enhances pulmonary host defenses in normal and ethanol-treated rats. J Infect Dis 164: 901–906.

Nelson S, Belknap SM, Carlson RW, Dale D, DeBoisblanc B, Farkas S, Fotheringham R, Ho H, Marrie T, Movahhed H, Root R & Wilson J, for the CAP Study Group (1998) A randomized controlled trial of filgrastim as an adjunct to antibiotics for treatment of hospitalized patients with community-acquired pneumonia. J Infect Dis 178: 1075–1080.

Nelson S, Heyder AM, Stone J, Bergeron MG, Daugherty S, Peterson G, Fotheringham N, Welch W, Milwee S & Root R (2000) A randomized controlled trial of filgrastim for the treatment of hospitalized patients with multilobar pneumonia. J Infect Dis 182: 970–973.

Neoptolemos JP, Wood P, Everson NW & Bell PRF (1985) Monocyte function following surgery in man. Increased numbers and stimulation of migration, phagocytosis and chemiluminescence following abdominal surgery. Eur Surg Res 17: 215–220.

Nicola NA, Metcalf D, Matsumoto M & Johnson GR (1983) Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. Identification as granulocyte colony-stimulating factor. J Biol Chem 258: 9017–9023.

Nicola NA, Begley CG & Metcalf D (1985) Identification of the human analogue of a regulator that induces differentiation in murine leukaemic cells. Nature 314: 625–628.

Nicola NA (1989) Hemopoietic cell growth factors and their receptors. Ann Rev Biochem 58: 45–77.

Nomura H, Imazeki I, Oheda M, Kubota N, Tamura M, Ono M, Ueyama Y & Asano S (1986) Purification and characterization of human granulocyte colony-stimulating factor (G-CSF). EMBO J 5: 871–876.

Novales JS, Salva AM, Modanlou HD, Kaplan DL, del Castillo J, Andresen J & Medlock ES (1993) Maternal administration of granulocyte colony-stimulating factor improves neonatal rat survival after lethal group B streptococcal infection. Blood 81: 923–927.

Ohsaka A & Saionji K (1998) In vivo administration of granulocyte colony-stimulating factor increases the surface expression of Sialyl-Lewis-X on neutrophils in healthy volunteers. Acta Haematol 100: 187–190.

Oka Y, Murata A, Nishijima J, Yasuda T, Hiraoka N, Ohmachi Y, Yasuda T, Kitagawa K, Toda H, Tanaka N, Ogawa M & Mori T (1994) Enhanced attachment and elastase-releasing capacity of neutrophils after surgery. Am J Surg 167: 405–411.

Okada Y, Kawagishi M & Kusaka M (1990) Effect of recombinant human granulocyte colony-stimulating factor on human neutrophil adherence in vitro. Experientia 46: 1050–1053.

Okrent DG, Lichtenstein AK & Ganz T (1990) Direct cytotoxicity of polymorphonuclear leukocyte granule proteins to human lung-derived cells and endothelial cells. Am Rev Respir Dis 141: 179–185.

Ono I, Gunji H, Zhang J-Z, Maruyama K & Kaneko F (1995) Studies on cytokines related to wound healing in donor site wound fluid. J Dermatol Sci 10: 241–245.

O'Reilly M, Silver GM, Greenhalgh DG, Gamelli RL, Davis JH & Hebert JC (1992) Treatment of intra-abdominal infection with granulocyte colony-stimulating factor. J Trauma 33: 679–682.

Osslund T & Boone T (1994) Biochemistry and structure of filgrastim (r-metHuG-CSF). In: Morstyn G & Dexter TM (eds) Filgrastim (r-metHuG-CSF) in clinical practice. Marcel Dekker inc. New York, p 23–31.

Oster W, Lindemann A, Mertelsmann R & Herman F (1989) Granulocyte-macrophage colony-stimulating factor (CSF) and multilineage CSF recruit human monocytes to express granulocyte CSF. Blood 73: 64–67.

Pajkrt D & van Deventer SJH (1997) Is G-CSF safe and useful in the treatment of infectious diseases in the non-neutropenic host? Intensive Care Med 23: 1–2.

Pajkrt D, Manten A, van der Poll T, Tiel-van Buul MMC, Jansen J, Wouter ten Cate J & van Deventer SJH (1997) Modulation of cytokine release and neutrophil function by granulocyte colony-stimulating factor during endotoxemia in humans. Blood 90: 1415–1424.

Patton JH, Lyden SP, Ragsdale DN, Groce MA, Fabian TC & Proctor KG (1998) Granulocyte colony-stimulating factor improves host defense to resuscitated shock and polymicrobial sepsis without provoking generalized neutrophil-mediated damage. J Trauma 44: 750–759.

Pearson CA, Pearson D, Shibara S, Hofsteenge J & Chiquet-Ehrismann (1988) Tenascin: cDNA cloning and induction by TGF-β. EMBO J 7: 2677–2981.

Perttilä J, Salo M & Rajamäki A (1987) Granulocyte microbicidal functions in patients undergoing major abdominal surgery under balanced anesthesia. Acta Anaesthesiol Scand 31: 100–103.

Pettengell R, Gurney H, Radford JA, Deakin DP, James R, Wilkinson PM, Kane K, Bentley J & Crowther D (1992) Granulocyte colony-stimulating factor to prevent dose-limiting neutropenia in non-Hodgin's lymphoma: a randomized controlled trial. Blood 80: 1430–1436.

Pettilä V, Takkunen O, Varpula T, Markkola A, Porkka K & Valtonen V (2000) Safety of granulocyte colony-stimulating factor (filgrastim) in intubated patients in the intensive care unit: interim analysis of a prospective, placebo-controlled, double-blind study. Crit Care Med 28: 3620–3625.

Philip MA, Standen G & Fletcher J (1980) The effects of surgical trauma on human granulopoiesis. Br J Haematol 44: 263–268.

Pitrak DL (1997) Effects of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor on the bactericidal functions of neutrophils. Curr Opin Hematol 4: 183–190.

Pollmächer T, Korth C, Mullington J, Schreiber W, Sauer J, Vedder H, Galanos C & Holsboer F (1996a) Effects of granulocyte colony-stimulating factor on plasma cytokine and cytokine receptor levels and on the in vivo host response to endotoxin in healthy men. Blood 87: 900–905.

Pollmächer T, Korth C, Schreiber W, Hermann D & Mullington J (1996b) Effects of repeated administration of granulocyte colony-stimulating factor (G-CSF) on neutrophil counts, plasma cytokine, and cytokine receptor levels. Cytokine 8: 799–803.

Preheim LC, Snitily MU & Gentry MJ (1996) Effects of granulocyte colony-stimulating factor in cirrhotic rats with pneumococcal pneumonia. J Infect Dis 174: 225–228.

Prockop DJ, Kivirikko KI, Tuderman L & Guzman NA (1979) The biosynthesis of collagen and its disorders. N Engl J Med 301: 13–23, 77–85.

Procopio MA, Rasias AJ, DeLeo JA, Pahl J, Hildebrandt L & Yeager MP (2001) The in vivo effects of general and epidural anesthesia on human immune function. Anesth Analg 93: 460–465.

Rajkovic JA & Williams R (1986) Abnormalities of neutrophil phagocytosis, intracellular killing, and metabolic activity in alcoholic cirrhosis and hepatitis. Hepatology 6: 252–262.

Repo H, Jansson S-E & Leirisalo-Repo M (1993) Flow cytometric determination of CD11b upregulation in vivo. J Immuno Methods 164: 193–202.

Rettig WJ, Erickson HP, Albino AP & Garin-Chesa P (1994) Induction of human tenascin (neuronectin) by growth factors and cytokines: cell type-specific signals and signalling pathways. J Cell Sci 107: 487–487.

Riedl S, Tandara A, Reinshagen M, Hinz U, Faissner A, Bodenmuller H, Buhr HJ, Herfarth C & Moller P (2001) Serum tenascin-C is an indicator of inflammatory bowel disease activity. Int J Colorectal Dis 16: 285–291.

Roilides E, Walsh TJ, Pizzo PA & Rubin M (1991) Granulocyte colony-stimulating factor enhances the phagocytic and bactericidal activity of normal and defective human neutrophils. J Infect Dis 163: 579–583.

Ruutu T & Kosunen TU (1972) In vitro effect of anti-inflammatory agents on phagocytosis and bacterial killing by human neutrophil leucocytes. Acta Pharmacol Toxocol 31: 226–237.

Ryhänen P, Surcel H-M & Ilonen J (1991) Decreased expression of class II major histocompatibility complex (MHC) molecules on monocytes is found in open-heart surgery related immunosuppression. Acta Anaesthesiol Scand 35: 453–456.

Sakagami H, Tajima M, Takayama F, Oi T, Kusama K, Yamamoto T, Saito M & Murayama J (2000) Role of carbohydrate moiety in granulocyte colony stimulating factor. Anticancer Res 20: 2355–2360.

Sakamaki S, Matsunaga T, Hirayama Y, Kuga T & Niitsu Y (1995) Haematological study of healthy volunteers 5 years after G-CSF. Lancet 346: 1432–1433.

Salo M, Perttilä J & Lehtonen O-P (1988) Granulocyte chemiluminescence in patients with postoperative infections. Arch Surg 123: 17–22.

Salo M (1992) Effects of anaesthesia and surgery on the immune response. Acta Anaesthesiol Scand 36: 201–220.

Schenk S, Muser J, Vollmer G & Chiquet-Ehrismann R (1995) Tenascin-C in serum: a questionable tumor marker? Int J Cancer 61: 443–449.

Schäfer H, Hübel K, Bohlen H, Mansmann G, Hegener K, Richarz B, Oberhäuser F, Wassmer G, Hölscher AH, Pichlmaier H, Diehl V & Engert A (2000) Perioperative treatment with Filgrastim stimulates granulocyte function and reduces infectious complications after esophagectomy. Ann Hematol 79: 143–151.

Selig C & Nothdurft W (1995) Cytokines and progenitor cells of granulocytopoiesis in peripheral blood of patients with bacterial infections. Infect Immun 63: 104–109.

Serushago BA, Yoshikai Y, Handa T, Mitsuyama M, Muramori K & Nomoto K (1992) Effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on murine resistance against Listeria monocytogenes. Immunology 75: 475–480.

Shigemitsu Y, Saito T, Kinoshita T & Kobayashi M (1992) Influence of surgical stress on bactericidal activity of neutrophils and complications of infection in patients with esophageal cancer. J Surg Oncol 50: 90–97.

Shimoda K, Okamura S, Omori F, Mizuno Y, Hara T, Aoki T, Ueda K & Niho Y (1991) Granulocyte colony-stimulating factor in cerebrospinal fluid from patients with meningitis. Blood 77: 2214–2217.

Shimoda K, Okamura S, Harada N, Kondo S, Okamura T & Niho Y (1993) Identification of a functional receptor for granulocyte colony-stimulating factor on platelets. J Clin Invest 91: 1310–1313.

Sietses C, Wiezer MJ, Eijsbouts QAJ, van Leeuwen PAM, Beelen RHJ, Meijer S & Cuesta MA (2000) The influence of laparoscopic surgery on postoperative polymorphonuclear leukocyte function. Surg Endosc 14: 812–816.

Silver GM, Gamelli RL & O'Reilly M (1989) The beneficial effect of granulocyte colony-stimulating factor (G-CSF) in combination with gentamicin on survival after Pseudomonas burn wound infection. Surgery 106: 452–456.

Simmers RN, Webber LM, Shannon MF, Garson OM, Wong G, Vadas MA & Sutherland GR (1987) Localization of the G-CSF gene on chromosome 17 proximal to the breakpoint in the t (15;17) in acute promyelocytic leukemia. Blood 70: 330–332.

Simpson DM & Ross R (1972) The neutrophilic leukocyte in wound repair. A study with antineutrophil serum. J Clin Invest 51: 2009–2023.

Smith JA (1994) Neutrophils, host defense, and inflammation: a double-edged sword. J Leuk Biol 56: 672–686.

Smith WC (2001) Neutrophils. In: Rich RR, Fleisher TA, Kotzin BL & Schroeder HW (eds) Clinical Immunology. Principles and practice. Mosby International Limited, London, p 22.1–22.15.

Souza LM, Boone TC, Gabrilove J, Lai PH, Zsebo KM, Murdock DC, Chazin VR, Bruszewski J, Lu H, Chen KK, Barendt J, Platzer E, Moore MAS, Mertelsmann R & Welte K (1986) Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. Science 232: 61–65.

Sprikkelman A, de Wolf JTM & Vellenga E (1994) The application of hematopoietic growth factors in drug-induced agranulocytosis: a review of 70 cases. Leukemia 8: 2031–2036.

Stahel RA, Jost LM, Cerny T, Pichert G, Honegger H, Tobler A, Jacky E, Fey M & Platzer E (1994) Randomized study of recombinant human granulocyte colony-stimulating factor after high-dose chemotherapy and autologous bone marrow transplantation for high-risk lymphoid malignancies. J Clin Oncol 12: 1931–1938.

Stanley TH, Hill GE, Portas MR, Hogan NA & Hill HR (1976) Neutrophil chemotaxis during and after general anesthesia and operation. Anesth Analg 55: 668–673.

Steed DL (1997) The role of growth factors in wound healing. In: Barbul A (ed) Wound healing. Surg Clin N Am 77: 575–587.

Stibenz D & Bührer C (1994) Downregulation of L-selectin surface expression by various leukocyte isolation procedures. Scand J Immunol 39: 59–63.

Stinner B, Bauhofer A, Lorenz W, Rothmund M, Plaul U, Torossian A, Celik I , Sitter H, Koller M, Black A, Duda D, Encke A, Greger B, van Goor H, Hanisch E, Hesterberg R, Klose KJ, Lacaine F, Lorijn RHW, Margolis C, Neugebauer E, Nyström PO, Reemst PHM, Schein M, Solovera J & Lucerne Group for Consensus-assisted Development of the Study Protocol on Prevention of Abdominal Sepsis: Example G-CSF (2001) Granulocyte-colony stimulating factor in the prevention of postoperative infectious complications and sub-optimal recovery from operation in patients with colorectal cancer and increased preoperative risk (ASA 3 and 4). Protocol of a controlled clinical trial developed by consensus of an international study group. Part three: individual patient, complication algorithm and quality management. Inflamm Res 50: 233–248.

Stolz DA, Bagby GJ & Nelson S (1997) Use of granulocyte colony-stimulating factor in the treatment of acute infectious diseases. Curr Opin Hematol 4: 207–212.

Sullivan KE, Snyder JR, Madigan JE, Pascoe JR, Farver TB, Thurmond MC & Andresen JW (1993) Effects of perioperative granulocyte colony-stimulating factor on horses with ascending colonic ischemia. Vet Surg 22: 343–350.

Takala AJ, Jousela IT, Takkunen OS, Jansson SR, Kyösola KT, Olkkola KT, Leirisalo-Repo M & Repo H (1996) Time course of β_2-integrin CD11b/CD18 (Mac-1, $\alpha_M\beta_2$) upregulation on neutrophils and monocytes after coronary artery bypass grafting. Scand J Thor Cardiovasc Surg 30: 141–148.

Tanaka H, Sugimoto H, Yoshioka T & Sugimoto T (1991) Role of granulocyte elastase in tissue injury in patients with septic shock complicated by multi-organ failure. Arch Surg 213: 81–85.

Tanaka H, Ishikawa K, Nishino M, Shimazu T & Yoshioka T (1996) Changes in granulocyte colony-stimulating factor concentration in patients with trauma and sepsis. J Trauma 40: 718–726.

Tanaka H, Tanaka Y, Shinagawa K, Yamagishi Y, Ohtaki K & Asano K (1997) Three types of recombinant human granulocyte colony-stimulating factor have equivalent biological activities in monkeys. Cytokine 9: 360–369.

Tepaske R, te Velthuis H, Oudemans-van Straaten HM, Heisterkamp SH, van Deventer SJH, Ince C, Eysman L & Kesecioglu J (2001) Effect of preoperative oral immune-enhancing nutritional supplement on patients at high risk of infection after cardiac surgery: a randomised placebo-controlled trial. Lancet 358: 696–701.

Toda H, Murata A, Matsuura N, Uda K, Oka Y, Tanaka N & Mori T (1993) Therapeutic efficacy of granulocyte colony stimulating factor against rat cecal ligation and puncture model. Stem Cells 11: 228–234.

Toda H, Murata A, Tanaka N, Ohashi I, Kato T, Hayashida H, Matsuura N, Monden M (1995) Changes in serum granulocyte colony-stimulating factor (G-CSF) and interleukin 6 (IL-6) after surgical intervention. Res Com Mol Pathol Pharmacol 87: 275–286.

Toft P, Nielsen CH, Tønnesen E, Hansen TG & Hokland M (1998) Changes in adhesion molecule expression and oxidative burst activity of granulocytes and monocytes during open-heart surgery with cardiopulmonary bypass compared with abdominal surgery. Eur J Anaesth 15: 345–353.

Toner GC, Green M, Bishop JF, McKendrick J, Cebon J, Sheridan WP, Lockbaum P, O'Byrne J & Fox RM (1998) Dose escalation study of carboplatin and cyclophosphamide with filgrastim support: a phase I study. Am J Clin Oncol 21: 263–269.

Tramont EC & Hoover DL (2000) Innate (general or nonspesific) host defence mechanisms. In: Mandell GL, Bennett JE & Dolin R (eds) Principles and practice of infectious diseases. Churchill Livingstone, Philadelphia, p 31–38.

Trillet-Lenoir V, Green J, Manegold C, Von Pawel J, Gatzemeier U, Lebeau B, Depierre A, Johnson P, Decoster G, Tomita D & Ewen C (1993) Recombinant granulocyte colony-stimulating factor reduces the infectious complications of cytotoxic chemotherapy. Eur J Cancer 29: 319–324.

Tsukada K, Katoh H, Shiojima M, Suzuki T, Takenoshita S & Nagamachi Y (1993) Concentrations of cytokines in peritoneal fluid after abdominal surgery. Eur J Surg 159: 475–479.

Tsukada K, Takenoshita S & Nagamachi Y (1994) Peritoneal interleukin-6, interleukin-8 and granulocyte elastase activity after elective abdominal surgery. APMIS 102: 837–840.

Udelsman R & Holbrook NJ (1994) Endocrine and molecular responses to surgical stress. Curr Probl Surg 31: 658–661.

Usui A, Kawamura M, Hibi M, Yoshida K, Murakami F, Tomita Y, Ooshima H & Murase M (1997) Blood concentrations of G-CSF and myelopoiesis in patients undergoing aortocoronary bypass surgery. Ann Hematol 74: 169–173.

Utoh J, Yamamoto T, Utsunomiya T, Kambara T, Goto H & Miyauchi Y (1988) Effects of surgery on neutrophil functions, superoxide and leukotriene production. Br J Surg 75: 682–685.

Vellenga E, Rambaldi A, Ernst TJ, Ostapovicz D & Griffin JD (1988) Independent regulation of M-CSF and G-CSF gene expression in human monocytes. Blood 71: 1529–1532.

Vial T & Descotes J (1995) Clinical toxicity of cytokines used as haemopoietic growth factors. Drug Safety 13: 371–406.

Villa P, Shaklee CL, Meazza C, Agnello D, Ghezzi P & Senaldi G (1998) Granulocyte colony-stimulating factor and antibiotics in the prophylaxis of a murine model of polymicrobial peritonitis and sepsis. J Infect Dis 178: 471–477.

Vincent ME, Foote M & Morstyn G (1994) Pharmacology of filgrastim (r-metHuG-CSF). In: Morstyn G & Dexter TM (eds) Filgrastim (r-metHuG-CSF) in clinical practice. Marcel Dekker inc. New York, p 33–50.

Wakefield CH, Carey PD, Foulds S, Monson JRT & Guillou PJ (1993) Polymorphonuclear leukocyte activation. An early marker of the postsurgical sepsis response. Arch Surg 128: 390–395.

Wang JM, Chen ZG, Colella S, Bonilla MA, Welte K, Bordignon C & Mantovani A (1988) Chemotactic activity of human recombinant granulocyte colony-stimulating factor. Blood 72: 1456–1460.

Waring PM, Presneill J, Maher DW, Layton JE, Cebon J, Waring LJ & Metcalf D (1995) Differential alterations in plasma colony-stimulating factor concentrations in meningococcaemia. Clin Exp Immunol 102: 501–506.

Watari K, Asano S, Shirafuji N, Kodo H, Ozawa K, Takaku F & Kamachi S (1989) Serum granulocyte colony-stimulating factor levels in healthy volunteers and patients with various disorders as estimated by enzyme immunoassay. Blood 73: 117–122.

Watts MJ, Addison I, Long SG, Hartley S, Warrington S, Boyce M & Linch DC (1997) Crossover study of the haematological effects and pharmacokinetics of glycosylated and non-glycosylated G-CSF in healthy volunteers. Br J Haematol 98: 474–479.

Weiss M, Gross-Weege W, Schneider M, Neidhardt H, Liebert S, Mirow N & Wernet P (1995) Enhancement of neutrophil function by in vivo filgrastim treatment for prophylaxis of sepsis in surgical intensive care patients. J Crit Care 10: 21–26.

Weiss M, Gross-Weege W, Harms B & Schneider EM (1996) Filgrastim (rhG-CSF) related modulation of the inflammatory response in patients at risk of sepsis or with sepsis. Cytokine 8: 260–265.

Welte K, Platzer E, Lu L, Gabrilove JL, Levi E, Mertelsmann R & Moore MAS (1985) Purification and biochemical characterization of human pluripotent hematopoietic colony stimulating factor. Proc Natl Acad Sci 82: 1526–1530.

Wenisch C, Werkgartner T, Sailer H, Patruta S, Krause R, Daxboeck F & Parschalk B (2000) Effect of preoperative prophylaxis with filgrastim in cancer neck dissection. Eur J Clin Invest 30: 460–466.

Whitby DJ, Longaker MT, Harrison MR, Adzick NS & Ferguson MWJ (1991) Rapid epithelialisation of fetal wounds is associated with the early deposition of tenascin. J Cell Sci 99: 583–586.

Wieser M, Bonifer R, Oster W, Lindemann A, Mertelsmann R & Herrmann F (1989) Interleukin-4 induces secretion of CSF for granulocytes and CSF for macrophages by peripheral blood monocytes. Blood 73: 1105–1108.

Wiezer MJ, Meijer C, Wallast-Groenewoud HP, Tool ATJ, Prins HA, Houdijk APJ, Beelen RHJ, Meijer S, Hack CE & van Leeuwen PAM (1999) Impaired leukocyte phagocytosis in patients undergoing hemihepatectomy for liver metastases. Liver Transpl Surg 5: 238–245.

Wilkinson PC (1982) Chemotaxis and inflammation. Churchill-Livingstone, Edinburgh, p 35–62.

Williams DA (2000) Stem cell model of hematopoiesis. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE & McGlave P (eds) Hematology. Basic principles and practice. Churchill Livingstone, Philadelphia, p 126–138.

Witte MB, & Barbul A (1997) General principles in wound healing. Surg Clin North Am 77: 509–528.

Wunderink RG, Leeper KV, Schein R, Nelson S, DeBoisblanc BP, Fotheringham N & Logan E (2001) Filgrastim in patients with pneumonia and severe sepsis or septic shock. Chest 119: 523–529.

Wyllie AH, Kerr JFR & Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68: 251–306.

Xu S, Höglund M & Venge P (1996) The effect of granulocyte colony-stimulating factor (G-CSF) on the degranulation of secondary granule proteins from human neutrophils in vivo may be indirect. Br J Hematol 93: 558–568.

Yalçin O, Soybir G, Köksoy F, Köse H, Öztürk R & Çokneseli B (1997) Effects of granulocyte colony-stimulating factor on bacterial translocation due to burn wound sepsis. Jpn J Surg 27: 154–158.

Yasuda H, Ajiki Y, Shimozato T, Kasahara M, Kawada H, Iwata M & Shimizu K (1990) Therapeutic efficacy of granulocyte colony-stimulating factor alone and in combination with antibiotics against Pseudomonas aeruginosa infections in mice. Infect Immun 58: 2502–2509.

Ylätupa S, Mertaniemi P, Haglund C & Partanen P (1995) Enzyme immunoassay for quantification of tenascin in biologic samples. Clin Biochem 28: 263–268.

Yokota K, Nishihira T, Shineha R, Sayama J, Nitta Y, Kimura M & Mori S (1995) Association between elevated plasma granulocyte colony-stimulating factor and the degree of surgical stress in patients undergoing gastrointestinal surgery. Jpn J Surg 25: 579–584.

Yong KL & Linch DC (1992) Differential effects of granulocyte- and granulocyte-macrophage colony-stimulating factors (G- and GM-CSF) on neutrophil adhesion in vitro and in vivo. Eur J Haematol 49: 251–259.

Yong K (1996) Granulocyte colony-stimulating factor (G-CSF) increases neutrophil migration across vascular endothelium independent of an effect on adhesion: comparison with granulocyte-macrophage colony-stimulating factor. Br J Hematol 94: 40–47.

Yoshino T, Tamura M, Hattori K, Kawamura A, Imai N & Ono M (1991) Effects of recombinant human granulocyte colony-stimulating factor on neutrophil function in normal rats. Int J Hematol 54: 455–462.

Yuo A, Kitagawa S, Ohsaka A, Ohta M, Miyazono K, Okabe T, Urabe A, Saito M & Takaku F (1989) Recombinant human granulocyte colony-stimulating factor as an activator of human granulocytes: potentiation of responses triggered by receptor-mediated agonists and stimulation of C3bi receptor expression and adherence. Blood 74: 2144–2149.

Yuo A, Kitagawa S, Ohsaka A, Saito M & Takaku F (1990) Stimulation and priming of human neutrophils by granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor: qualitative and quantitative differences. Biochem Biophys Res Commun 171: 491–497.

Zarco MA, Ribera JM, Urbano-Ispizua A, Filella X, Arriols R, Martínez C, Feliu E & Montserrat E (1999) Phenotypic changes in neutrophil granulocytes from healthy donors after G-CSG administration. Haematologica 84: 874–878.

Zhan Y, Lieschke GJ, Grail D, Dunn AR & Cheers C (1998) Essential roles for granulocyte-macrophage colony-stimulating factor (GM-CSF) and G-CSF in the sustained hematopoietic response of Listeria monocytogenes-infected mice. Blood 91: 863–869.

Zsebo KM, Cohen AM, Murdock DC, Boone TC, Inoue H, Chazin VR, Hines D & Souza LM (1986) Recombinant human granulocyte colony stimulating factor: molecular and biological characterization. Immunobiol 172: 175–184.

Zsebo KM, Yuschenkoff VN, Schiffer S, Chang D, McCall E, Dinarello CA, Brown MA, Altrock B & Bagby GC (1988) Vascular endothelial cells and granulopoiesis: interleukin-1 stimulates release of G-CSF and GM-CSF. Blood 71: 99–103.