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

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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

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Oulu, September 2002

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

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


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).

## Contents

Abstract

Acknowledgements

Abbreviations

List of original publications

1 Introduction ................................................. 15

2 Review of the literature ................................. 17

2.1 Pathophysiology of acute inflammation ............... 17

2.1.1 General ............................................. 17

2.1.2 Leukocyte-tissue interaction ....................... 18

2.1.3 Neutrophils at the site of inflammation .......... 19

2.2 Acute inflammation triggered by surgery and anaesthesia ............ 21

2.2.1 General ............................................. 21

2.2.2 Cytokine response to surgery ....................... 22

2.2.3 Leukocyte response to surgery ..................... 23

2.3 Hematopoiesis ........................................... 25

2.4 Granulocyte colony-stimulating factor (G-CSF) ........ 26

2.4.1 General ............................................. 26

2.4.2 In vitro and in vivo activities ....................... 27

2.4.3 Recombinant forms (rhG-CSFs) ..................... 27

2.4.4 Clinical applications of rhG-CSF .................... 28

2.4.5 Adverse effects of rhG-CSF ......................... 29

2.4.6 In infectious diseases ............................... 29

2.4.6.1 Experimental models ........................... 30

2.4.6.2 In humans ....................................... 31

2.4.7 In surgery ........................................... 31

2.5 Wound healing and extracellular matrix .............. 32

2.5.1 General principles ................................. 32

2.5.2 Collagens .......................................... 33

2.5.3 Tenascin ........................................... 33

3 Aims of the study ........................................ 35

4 Material and methods .................................. 36

4.1 Clinical methods ...................................... 36

4.2 Ethical considerations ................................. 36
4.3 Surgery and anaesthesia .............................................. 37
4.4 Study medication ..................................................... 37
4.5 Collection of samples ................................................ 37
4.6 Leukocyte counts ..................................................... 37
4.7 Neutrophil chemotaxis, phagocytosis and microbicidal activity .... 38
4.8 Neutrophil and monocyte phagocytosis and respiratory burst .......... 39
4.9 Neutrophil adhesion molecules .................................... 39
4.10 Tests on cytokines and C-reactive protein (CRP) .................... 40
4.11 Tests on procollagen propeptides and tenascin-C (Tn-C) ............. 40
4.12 Statistical methods .................................................. 40
5 Results .............................................................. 42
  5.1 First trial .......................................................... 42
    5.1.1 General ....................................................... 42
    5.1.2 Leukocytes .................................................... 42
    5.1.3 Neutrophil functions ....................................... 43
    5.1.4 Neutrophil adhesion molecules ............................ 44
    5.1.5 CRP and cytokine levels .................................. 44
  5.2 Second trial ....................................................... 45
    5.2.1 Leukocytes .................................................... 45
    5.2.2 CRP and cytokine levels .................................. 46
  5.3 Third trial .......................................................... 46
    5.3.1 Procollagen propeptide concentrations .................... 46
    5.3.2 Tn-C levels .................................................. 46
6 Discussion .......................................................... 47
  6.1 Methodological considerations ................................... 47
  6.2 Filgrastim ......................................................... 48
  6.3 Differences between the body compartments ........................ 48
  6.4 Effects of surgery on leukocytes ................................ 49
  6.5 Effects of surgery on cytokines .................................. 49
  6.6 Postoperative procollagen propeptides and Tn-C ..................... 49
  6.7 Future ............................................................. 50
7 Conclusions .......................................................... 51
References
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, Rolides 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 adrenocorticotrophic 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).
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 (Kuhs 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.

<table>
<thead>
<tr>
<th>Granule</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary (azurophilic)</td>
<td>myeloperoxidase, elastase, cathepsin G, proteinase 3, lysozyme, α-mannosidase, β-glucuronidase, β-glycerophosphatase, sialidase</td>
</tr>
<tr>
<td>Secondary (specific)</td>
<td>lactoferrin, lysozyme, gelatinase, histaminase, sialidase</td>
</tr>
<tr>
<td>Tertiary</td>
<td>lysozyme, gelatinase, acetyltransferase</td>
</tr>
<tr>
<td>Secretory vesicles</td>
<td>alkaline phosphatase</td>
</tr>
</tbody>
</table>

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 sequelae 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.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Source</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>Monocyte/macrophage, T-lymphocyte</td>
<td>↑ 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</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Monocyte/macrophage, T-lymphocyte, natural killer cell, endothelial cell, epithelial cell, keratinocyte, fibroblast, dendritic cell</td>
<td>↑ 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</td>
</tr>
<tr>
<td>IL-2</td>
<td>T-lymphocyte</td>
<td>↑ overall immunocompetence, ↑ cytotoxic T-lymphocyte proliferation, ↑ reticuloendothelial system activity, ↑ gut barrier immunity</td>
</tr>
<tr>
<td>IL-6</td>
<td>Monocyte/macrophage, T-lymphocyte, fibroblast, endothelial cell</td>
<td>↑ fibroblast antiviral activity, ↑ lymphocyte differentiation, ↑ B-lymphocyte immunoglobulin production, ↑ hepatocyte acute-phase protein production, ↑ prostaglandin production,</td>
</tr>
<tr>
<td>IL-8</td>
<td>Monocyte/macrophage, T-lymphocyte, endothelial cell, platelet</td>
<td>↑ chemotaxis of neutrophils, macrophages and lymphocytes</td>
</tr>
<tr>
<td>IL-10</td>
<td>B-lymphocyte, T-lymphocyte</td>
<td>↓ inflammatory cytokine synthesis by monocyte/macrophages and lymphocytes, modulates inflammatory activities of TNF-α, IL-1β, IL-6, IL-8 and IFN-γ</td>
</tr>
<tr>
<td>IL-12</td>
<td>Monocyte/macrophage, neutrophil, keratinocyte, dendritic cell</td>
<td>Stimulates T-lymphocytes, ↑ lymphocyte and natural killer cell production, ↑ B-lymphocyte immunoglobulin production, ↑ haematopoiesis,</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Monocyte/macrophage, T-lymphocyte, natural killer cell</td>
<td>↑ 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</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>T-lymphocyte, fibroblast, endothelial cell, stromal cell</td>
<td>↑ myeloproliferation of monocytes, neutrophils and eosinophils, ↑ chemotaxis of neutrophils and monocyte/macrophages, ↑ cytokine production by monocyte/macrophages</td>
</tr>
</tbody>
</table>
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 3. Studies examining cytokine concentrations locally at the operative site. PF; peritoneal fluid, WF; wound fluid, DR; drainage fluid, \( \rightarrow \); positive correlation.

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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of surgery (no. of patients) and main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cullen et al. 1975</td>
<td>Elective surgery (18). Neutrophil and monocyte phagocytosis ↓, NBT reduction ↓ after anaesthesia</td>
</tr>
<tr>
<td>Stanley et al. 1976</td>
<td>Elective orthopedic, gynaecologic, plastic or neurosurgical operations (43). Neutrophil chemotaxis ↓ after anaesthesia, but was restored by surgery</td>
</tr>
<tr>
<td>Bowers et al. 1977</td>
<td>Healthy kidney donors (25). Neutrophil phagocytosis ↔, killing ↔, chemotaxis ↓ 1 h, ↔ 1 d, ↑ 2 d, adherence ↓ 1 h, ↔ 1 d, ↔ 2 d after surgery</td>
</tr>
<tr>
<td>Philip et al. 1980</td>
<td>Abdominal hysterectomy (12). Neutrophil colony-forming units in culture (CFUc) ↓ 1 d, ↑ 7 d, ↔ 15 d after surgery,</td>
</tr>
<tr>
<td>Moudgil et al. 1981</td>
<td>Elective plastic, orthopedic and neurosurgical operations (30). Neutrophil chemotaxis ↓ immediately, ↓ 24 h, and ↓ 48 h after surgery, and was restored 72 h after surgery</td>
</tr>
<tr>
<td>El-Maallem &amp; Fletcher 1981</td>
<td>Abdominal hysterectomy (31). Neutrophil phagocytosis ↔, killing ↓ 24 h, ↓ 72 h, ↔ restored 8 d after surgery</td>
</tr>
<tr>
<td>Endler et al. 1982</td>
<td>Hip arthroplasty (14). Neutrophil chemotaxis ↓ 1 d, ↑ 3 d, ↔ 6 d after surgery</td>
</tr>
<tr>
<td>Van Dijk et al. 1982</td>
<td>&quot;Major surgical procedures&quot; (48). Neutrophil chemotaxis ↔, phagocytosis ↔, chemiluminescence ↔ 24 h, and 48 h after surgery</td>
</tr>
<tr>
<td>Davies et al. 1983</td>
<td>Abdominal hysterectomy (10). Neutrophil β-glucuronidase activity ↔ 4 h, ↓ 24 h, ↔ 5 d, lysozyme activity ↓ 4 h, ↓ 24 h, ↔ 5 d, B12-binding capacity ↓ 4 h, 24 h, ↔ 5 d after surgery</td>
</tr>
<tr>
<td>Mealy et al. 1987</td>
<td>Elective general surgery (13). Neutrophil chemiluminescence ↓ after anaesthesia, ↑ 24 h after surgery</td>
</tr>
<tr>
<td>Perttilä et al. 1987</td>
<td>Elective major abdominal surgery (11). Neutrophil chemiluminescence ↔ 1-7 d, proportion of high-peroxidase-activity neutrophils ↑ 1 d, ↑ 3-4 d, ↔ 6-7 d after surgery</td>
</tr>
<tr>
<td>Krausz et al. 1988</td>
<td>Elective surgical procedures (20). Neutrophil random migration and chemotaxis ↔ 24 h, thromboxane B2-production ↓ 24 h after surgery</td>
</tr>
<tr>
<td>Salo et al. 1988</td>
<td>General surgery (17). Neutrophil chemiluminescence ↓ in patients with major postoperative infections</td>
</tr>
<tr>
<td>Utoh et al. 1988</td>
<td>Open heart surgery, abdominal surgery (28). Neutrophil superoxide production ↓, LTBP4 production ↑, LTC4 production ↓ 1 d after surgery</td>
</tr>
<tr>
<td>Shigemitsu et al. 1992</td>
<td>Gastrointestinal surgery (50). Neutrophil intracellular killing ↓, superoxide anion production ↓ 1 d, myeloperoxidase activity ↑ 1-3 d after surgery</td>
</tr>
<tr>
<td>Wakefield et al. 1993</td>
<td>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)</td>
</tr>
<tr>
<td>Oka et al. 1994</td>
<td>Gastrointestinal surgery (14). Neutrophil attachment ↑, elastase-releasing capacity ↑ after surgery. In patients with complications the rise was long-lasting</td>
</tr>
<tr>
<td>Jensen et al. 1995</td>
<td>Open heart surgery (12). Neutrophil chemotaxis ↓ 1 d after surgery</td>
</tr>
<tr>
<td>Khan et al. 1995</td>
<td>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</td>
</tr>
</tbody>
</table>
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.

Table 4. (Continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of surgery (no. of patients) and main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takala et al. 1996</td>
<td>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.</td>
</tr>
<tr>
<td>Barry et al. 1997</td>
<td>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.</td>
</tr>
<tr>
<td>Klava et al. 1997</td>
<td>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.</td>
</tr>
<tr>
<td>Toft et al. 1998</td>
<td>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.</td>
</tr>
<tr>
<td>Sietses et al. 2000</td>
<td>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.</td>
</tr>
</tbody>
</table>
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 tumors 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 arachidonic 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 (Böning 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 Auwerda 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, Morstyn et al. 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 methionine, 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).

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


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, 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- \(\alpha\) (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 functions 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.


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.

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

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 Tenasin

Tenasin is an extracellular matrix glycoprotein also known as myotendinous antigen, glioma mesenchymal ECM protein, hexabrachion, brachionectin, J1 and cytactin (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).

Tenasin (Tn)-C is the most widely studied member of the tenasin 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 PIINP (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>
<thead>
<tr>
<th>Trial</th>
<th>Surgery (n)</th>
<th>Study drug</th>
<th>Samples</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>colorectal surgery, randomized, blinded trial (30)</td>
<td>filgrastim vs. placebo</td>
<td>blood, peritoneal fluid, wound fluid</td>
<td>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</td>
</tr>
<tr>
<td>2</td>
<td>colorectal surgery (18)</td>
<td>none</td>
<td>blood</td>
<td>leukocyte counts, neutrophil and monocyte phagocytosis and respiratory burst, CRP, IL-6, IL-8, G-CSF</td>
</tr>
<tr>
<td>3</td>
<td>major abdominal surgery (24)</td>
<td>none</td>
<td>blood, wound fluid</td>
<td>PINP, PIIINP, Tn-C</td>
</tr>
</tbody>
</table>

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 Polymorphprep (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 neutrophil s 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 10⁶/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 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 10⁶ 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 10⁶ 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 15 pg/mL for TNF-α, 4 pg/mL for IL-6, 15 pg/mL for IL-8, 62.5 pg/mL for TGF-β, 2 pg/mL for IL-1β and 1 pg/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 immunoassay (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 4 pg/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 25th and 75th 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.

<table>
<thead>
<tr>
<th></th>
<th>Filgrastim group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phagocytosis (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>78.1 (± 11.9)</td>
<td>72.4 (± 9.9)</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>69.0 (± 13.8)</td>
<td>69.1 (± 12.3)</td>
</tr>
<tr>
<td>Wound fluid</td>
<td>78.4 (± 5.0)</td>
<td>67.3 (± 9.0)</td>
</tr>
<tr>
<td><strong>Phagocytosis index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>2.9 (± 0.7)</td>
<td>2.6 (± 0.5)</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>2.4 (± 0.7)</td>
<td>2.5 (± 0.7)</td>
</tr>
<tr>
<td>Wound fluid</td>
<td>2.8 (± 0.2)</td>
<td>2.4 (± 0.6)</td>
</tr>
<tr>
<td><strong>Killed bacteria (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>26.8 (± 11.2)</td>
<td>32.8 (± 14.9)</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>26.2 (± 14.4)</td>
<td>29.5 (± 14.2)</td>
</tr>
<tr>
<td><strong>Killed bacteria (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>56.0 (± 8.0)</td>
<td>50.6 (± 13.4)</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>51.4 (± 6.5)</td>
<td>49.6 (± 8.8)</td>
</tr>
</tbody>
</table>

**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-C levels

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

The healing of laparotomy wounds did not differ between the study groups. The classic study with anti-neutrophil serum showed that, in the absence 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.

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 PIINP 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 disappointing. 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


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