Pleiotropic responses to interferon-gamma in humans

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Citation for published version (APA):
Chapter 8

Interferon-gamma administration after abdominal surgery rescues antigen-specific helper T-cell immune reactivity

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Submitted for publication
Abstract

Antigen induced activation of T cells is determined by 1) the number of T cell receptors (TCR's) triggered by TCR-ligands on antigen presenting cells (APC's), and 2) the intrinsic cellular threshold for activation. T cell receptor triggering is optimized by adhesion molecules, that form the interaction site between T cells and APC's, i.e. the immunological synapse. In addition, signals through costimulatory molecules lower the intrinsic T cell activation threshold. Immunosuppressive agents and traumatic events such as major operative procedures change physiological T cell responses. Depressed immune functions after surgery are presumed to render patients more susceptible to pathogens. Interferon-gamma (IFN-γ) is a type II homodimeric cytokine with multiple immunostimulatory properties. Several studies have been performed to assess the effects of IFN-γ treatment in patients in need of increased immune reactivity. However, until now, the effect of IFN-γ on human antigen specific CD4⁺ T cell reactivity after surgically induced immunosuppression has not been reported. Therefore, a comparative trial of recombinant human (rh)IFN-γ versus placebo in patients after abdominal surgery was initiated. Antigen-specific helper T cell immune reactivity was assessed by antigen induced cytokine production, intracellular cytokine staining and flow cytometry. A single dose of rhIFN-γ rescued downmodulation of antigen specific CD4⁺ T cell reactivity, concomitant with an upregulation of TCR-ligands on antigen presenting cells. Selected patients may benefit from the immunostimulatory properties of rhIFN-γ administration in vivo.
Introduction

The clinical outcome of infections is not only determined by the nature and dose of infectious agents but also by host resistance mechanisms. The host defense against pathogenic microorganisms is disturbed after severe trauma, and surgical stress is similarly capable of inducing immunosuppressive effects (1-6). After surgical trauma, densities of HLA class II molecules on monocytes are dramatically reduced and thereby the number of potential TCR-ligands for CD4+ T cells is diminished (7-9).

Administration of recombinant human IFN-γ has been performed in severe burn patients and trauma patients. Although a decline of infection rate or death rate upon IFN-γ administration was not found in all patients, subgroups of trauma or burn patients are believed to experience beneficial effects from this treatment (10-13). Moreover, administration of rhIFN-γ does reduce the frequency and severity of infections in patients with chronic granulomatous disease (14).

IFN-γ exerts various effects on the immune system in vivo. First, IFN-γ depresses peripheral blood leukocyte counts, presumably by redistribution of cells from the circulation to secondary lymphoid organs and solid tissues (15,16). Second, IFN-γ increases Fcγ-receptor-1 (FcγRI) expression on neutrophilic granulocytes, thereby increasing the Fc mediated, but not complement mediated phagocytosis of bacteria (15,17). Third, IFN-γ increases FcγRII and III- and integrin expression on monocytes and hydrogen peroxide production by monocytes (10,15). Fourth, it has been demonstrated that IFN-γ augments levels of lipopolysaccharide-binding protein (15). Finally, after surgical interventions, IFN-γ reverses depressed HLA class II expression on monocytes (18). Thereby, densities of TCR-ligands for CD4+ T cells is reestablished at high levels on cells that differentiate into antigen presenting cells (19). It has not been previously resolved whether these effects of IFN-γ elevate antigen specific T cell immune reactivity in vivo in humans. Recently developed tools allow determination of frequency and phenotype of antigen specific CD4+ T cells from human peripheral blood (20-23). These assays depend on antigen induced CD4+ T cell activation and production of effector cytokines, intracellular cytokine staining and flowcytometric analysis. We performed a comparative, placebo controlled study into the effects of rhIFN-γ on
antigen-specific immunity of patients after a pylorus-preserving pancreaticoduodenectomy. During a study period of two days after rhIFN-γ administration, characteristics of antigen-specific CD4⁺ T cell reactivity were studied simultaneously with properties of circulating monocytes. By evaluating responses to cytomegalovirus (CMV) and Staphylococcus Aureus Enterotoxin B (SEB), we analyzed whether administration of rhIFN-γ can rescue antigen specific CD4⁺ T cell reactivity after surgical trauma, by stimulating antigen presenting cell functionality.

Materials and methods

Patients
Patients, scheduled for elective pylorus-preserving pancreaticoduodenectomy aimed at curative treatment of a suspicious tumor in the pancreatic head, papilla of Vater, distal bile duct or duodenum were eligible for entry into this study. CMV-seronegative patients do not have detectable frequencies of CMV-specific CD4⁺ lymphocytes in peripheral blood (20,23) and therefore, CMV-seronegative patients were excluded from this study. Other exclusion criteria were: 1) jaundice at hospital admission (bilirubin levels exceeding 40 \( \mu \text{mol/L} \), preoperative biliary drainage was accepted); 2) fever in the period of two weeks prior to hospital admission; 3) irresectability as a peroperative finding, and therefore deviation of the intended pylorus-preserving pancreaticoduodenectomy procedure towards a bypass procedure; 4) Clinical instability or evidence of infection on the day of the study (second post-operative day). All patients gave written informed consent, in accordance with the Helsinki Declaration of Human Rights. The study was approved by the Research Committee and the Medical Ethical Committee of the Academic Medical Center, Amsterdam, The Netherlands.

Study design
One day prior to the surgical procedure, patients were asked to consent with the study, under the condition of being CMV-seropositive. The study design is schematically drawn in figure 1. Blood was sampled for determination of CMV serostatus and routine biochemical and hematological measurements. Two days
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**Figure 1** Design of the study: Blood collections are indicated by arrows. Blood collections were performed on admission for CMV serology; at day 0, just before administration of rhIFN-γ or placebo, and at 24 and 48 hours thereafter for determination of monocytes, lymphocyte subsets and CMV- and SEB-specific CD4+ T cell reactivity.

after surgery and just before administration of recombinant human (rh) IFN-γ or placebo, blood was drawn for determination of monocytes, lymphocyte subsets and CMV- and SEB-specific CD4+ T cells. At 09.00 A.M., rhIFN-γ (100 μg/m², Immukine, Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) or an equivalent volume of saline solution was injected subcutaneously in the upper leg. Twenty-four and 48 hours after the injection of rhIFN-γ or saline, blood was drawn, again for determination of monocytes, lymphocyte subsets and CMV- and SEB-specific CD4+ T-cells.

**Anti-CMV antibodies**

Before surgery, anti CMV IgG was determined in serum using the AxSYM micro particle enzyme immunoassay (Abbott Laboratories, Abbott Park, Illinois, USA) according to the manufacturer’s instructions.

Immunofluorescence and flow-cytometry

EDTA-anticoagulated whole blood was directly cooled on ice. Whole blood was fixed in 0.5% (w/v) paraformaldehyde (PFA) for 5 minutes at 4°C. Subsequently, erythrocytes were lysed twice in ammonium chloride (0.155 M) in water containing 0.5 mM potassium EDTA. Leukocytes were refixed in 2% PFA and washed in PBS (phosphate buffered saline) containing 5% (w/v) Bovine Serum Albumin (BSA, Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands), 0.01% (w/v) NaN₃ and potassium EDTA (PBAP). Aspecific binding of antibodies was blocked by addition of 10% (v/v) human pooled serum (HPS, BioWhittaker Inc. Walkersville, Maryland, USA) in PBAP. Cells were incubated with fluorescent
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label conjugated primary antibodies for 30 minutes at 4°C protected from light. Subsequently, cells were washed and resuspended in PBAP and analyzed on a dual laser FACS Calibur flow cytometer equipped with a 488 nm argon ion laser and a 635nm red diode laser. On day 0, photomultiplier tube (PMT) voltage- and electronic compensation adjustments were performed using unstained cells or cells, single stained with either CD8-FITC, CD8-PE or CD8-PerCP (BectonDickinson Immunocytometry Systems, San Jose, California, USA(BD)). At day 1 and 2 after rhIFN-γ or placebo treatment PMT voltages and electronic compensation from day 0 zero were used (but checked for accuracy). Other monoclonal antibodies used were CD45RA-FITC, CD27-PE, CD4-PerCP (all BD), anti-HLA class II-FITC (Dako, Glostrup, Denmark). For determination of the costimulatory molecules CD80 (B7.1) and CD86 (B7.2) a two step staining protocol was followed. After addition of HPS, the peripheral blood leukocytes were incubated with CD80 (M24) or CD86 (1G10, both kindly provided by Dr. M. de Boer, Tanox Pharma B.V. Amsterdam, The Netherlands), for 30 minutes at 4°C protected from light. Subsequently, cells were washed in PBAP and stained with the secondary antibody goat anti mouse immunoglobulin-FITC (1299, CLB, Amsterdam, The Netherlands) for 30 minutes at 4°C protected from light, washed and analyzed on a FACS Calibur flow cytometer.

Data files containing 30,000 events within a lymphocyte gate were saved. Frequencies of CD45RA<sup>+</sup>CD27<sup>+</sup> cells within gated CD4<sup>+</sup> lymphocytes were determined using Cellquest software (BD) and designated naive CD4<sup>+</sup> T cells (24-26). The CD45RA<sup>-</sup>CD27<sup>-</sup>CD4<sup>+</sup> and CD45RA<sup>-</sup>CD27<sup>-</sup>CD4<sup>+</sup> T cell frequencies were added and designated memory CD4<sup>+</sup> T cells. For HLA-class II expression levels on monocytes, mean fluorescence intensities (MFI) were determined in the monocyte gate. Because expression of CD80 and CD86 on monocytes does not clearly exceed background levels, MFI’s were normalized to control antibody MFI.

**Determination of antigen specific CD4<sup>+</sup> T cells**

CMV- and SEB-specific CD4<sup>+</sup> T cell frequencies were determined according to the method described by Suni et al. (22). Briefly, 1 ml of heparin-anticoagulated whole blood was incubated for 6 hours in the presence of either CMV antigen (BioWhittaker, 60 μl/ml), control antigen (BioWhittaker, 60 μl/ml, negative control), no antigen (additional negative control) or Staphylococcus Aureus
enterotoxin B (SEB, Fluka, 1μg/ml), in 15 ml polypropylene tubes (Falcon labware, BD). CD28 monoclonal antibody (mAb) (clone 15E8, CLB, The Netherlands) was added as 3 μg/ml (final concentration). For the final 5 hours of culture, brefeldin A (Sigma-Aldrich Chemie BV) was added to the cultures in a final concentration of 10 μg/ml. In a subgroup of patients, CMV-specific CD4+ T cell frequency was also determined in the absence of CD28 antibody incubations. After 6 hours, 100 μL per tube of 20mM potassium EDTA in PBS was added to the cultures and the tubes were incubated at room temperature for 15 minutes. Erythrocytes were lysed by addition of 10 ml per tube FACS lysing solution (BD) and incubation for 10 minutes at room temperature. Cells were pelleted and resuspended in 0.5 ml per tube FACS permeabilizing solution and incubated for 10 minutes at room temperature. Three ml of PBS containing 5%(w/v) BSA and 0.01% (w/v) NaN₃ (PBA) was added, and cell suspensions were transferred to FACS-tubes and pelleted. Cells were stained with CD4-APC(BD), CD69-PE (BD) and anti- IFN-γ-FITC (BD) according to the manufacturers’ instructions for 30 minutes at 4°C protected from light. Subsequently, cells were washed in PBA. Cells were analyzed on a FACS Calibur flow cytometer, as previously described. Data files containing 50,000 events positive for CD4-APC fluorescence within a lymphocyte gate were saved. Frequencies of CD69+ IFN-γ cells within the CD4+ lymphocyte gate were determined using Cellquest software (BD) and designated CMV-specific CD4+ T cell frequency in case of CMV stimulated cultures or SEB-specific CD4+ T cells in case of SEB stimulated cultures.

Statistical analysis
Changes from baseline (i.e. the measurement at day 0, two days after surgery) per patient were calculated. Within each treatment group, the change from baseline of continuous numerical variables was analyzed using Wilcoxon’s signed rank test. Differences in changes from baseline between treatment groups were analyzed by the Mann-Whitney test. Tests were performed using SPSS 7.5.3 software (SPSS Inc., Chicago, Illinois, USA)
Table 1: Patient characteristics

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<th>Placebo</th>
<th>IFN-γ</th>
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<th>ns (Mann-Whitney)</th>
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<td>Sex (female/male)</td>
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<td>1/5</td>
<td>ns (Fishers exact)</td>
<td>ns (Mann-Whitney)</td>
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<td>Median age at day 0 in years (range)</td>
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<td>64(48-77)</td>
<td>ns (Mann-Whitney)</td>
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<td>Median duration of anaesthesia in minutes (range)</td>
<td>285(210-330)</td>
<td>367.5(155-425)</td>
<td>ns (Mann-Whitney)</td>
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</table>

Fig. 2a

Before IFN-γ day1 day2

FITC- fluorescence (arbitrary units)

Control anti HLA-class II-FITC

Fig. 2b

Figure 2 Administration of rhIFN-γ but not placebo induces monocyte HLA class II expression:

a.) Histograms of monocytes (gated on forward scatter and sideward scatter parameters) with respect to background fluorescence (dashed lines) or anti-HLA-class II-FITC fluorescence (solid lines) (X-axis, arbitrary units, log scale) versus relative cell number (Y-axis). Histograms are from one representative patient treated with rhIFN-γ and represent measurements obtained just before administration of rhIFN-γ (day 0, left panel), 24 hours after administration of rhIFN-γ (day 1, middle panel) and 48 hours after administration of rhIFN-γ (day 2, right panel).

b.) Time (in days after administration of rhIFN-γ or placebo, X-axis) versus HLA-class II expression on monocytes (in mean fluorescence intensity (arbitrary units), Y-axis) of rhIFN-γ (closed circles) and placebo- (open circles) treated patients. Circles re-present means, error bars represent the standard error of the mean.
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Results

Patients
From December 1998 until February 2000, 12 patients were included into this study. Six patients were assigned to rhIFN-γ treatment and six patients received placebo, both at day 2 after surgery. Patients in both groups were comparable with respect to sex, age at entry into the study and mean duration of the surgical procedure (table 1).

Expression of HLA-class II on peripheral blood monocytes
Before treatment, HLA class II expression on monocytes was low. In placebo treated patients, HLA class II expression remained low at the first day of follow up. At the second day after administration of placebo, a small, statistically not significant increase in MFI for HLA class II was observed (figure 2b). In accordance with previous reports, treatment with rhIFN-γ dramatically increased expression of HLA class II on monocytes (figure 2a and b) (10,11,15,18). Thus, administration of rhIFN-γ increases densities of potential T cell receptor-ligands on antigen presenting cells.

CMV-specific and SEB-specific CD4+ T cell reactivity in peripheral blood is rescued by rhIFN-γ treatment
Figure 3 shows the CMV- and SEB-specific CD4+ T cell frequencies from one representative patient two days after surgery and just before administration of rhIFN-γ. In cultures incubated either without antigen (not shown) or with control antigen, no IFN-γ-producing cells are found (figure 3 upper left panel). Treatment with rhIFN-γ did not increase the frequencies of cells positive for IFN-γ staining after incubation without antigen or with control antigen (not shown). Therefore, occupancy of IFN-γ-receptors by rhIFN-γ is not detectable by flow cytometry in peripheral blood derived CD4+ T cells at 24 hours or 48 hours after rhIFN-γ administration in these patients. Moreover, plasma levels of IFN-γ are back to normal 24 hours after rhIFN-γ administration (16). In cultures incubated with CMV antigen, a small but clearly distinguishable CD4+ T cell population became detectable consisting of activated (CD69+), IFN-γ producing cells (figure 3, second
Before day1 day2

IFN-γ

control Ag with CD28

CMV Ag no CD28

CMV Ag with CD28

SEB with CD28

row of panels). This population is absent in individuals who are CMV-seronegative and virtually disappears if TCR/MHC interactions are blocked by co-incubation of

Figure 3
CMV-specific and SEB-specific CD4+ T cell reactivity:
The dotplots represent CD4+ lymphocytes with respect to anti-IFN-γ-FITC fluorescence (X-axis, arbitrary units, log scale) versus CD69 fluorescence (Y-axis, arbitrary units, log scale). The dotplots represent cells from one rhIFN-γ-treated, CMV-seropositive patient. Flow cytometric analysis was performed after incubation with control antigen (upper panels), CMV antigen in the absence (second row of panels) or presence of CD28 monoclonal antibody (third row of panels) or Staphylococcus Aureus Enterotoxin B (SEB, lower panels).
Analyses were performed just before administration of IFN-γ (left column of panels), 24 hours (middle column of panels) or 48 hours after administration of rhIFN-γ (right column of panels).
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the cultures with MHC class II reactive antibodies (20-22). Therefore, this population of activated cells is considered to be antigen specific. Although activated in an antigen specific and MHC class II restricted manner, the number of these cells increases by addition of co-stimulatory signals. Accordingly, addition of CD28 antibodies to the CMV stimulated cultures increased the frequency of activated cells producing IFN-γ (compare figure 3 second row of panels and third row of panels). This increase in the frequency of antigen specific CD4+ T cells by CD28 is likely due to a lowering of the activation threshold for IFN-γ production by cells that would not have been sufficiently triggered in the absence of additional costimulatory signals (20,21,27).

CD4+ T cell reactivity to CMV depends on uptake of antigen, antigen processing and peptide loading on MHC class II molecules by antigen presenting cells, activities that may be differentially regulated by IFN-γ. SEB, being a superantigen, directly triggers T cells by crosslinking T cell receptor β-chain variable regions to MHC class II molecules, a process which is independent from antigen processing or peptide loading (28,29). The lower panels of figure 3 show the frequency of SEB-reactive CD4+ T cells from one representative patient before administration of rhIFN-γ. Generally, the frequencies of SEB-reactive CD4+ T cells was found to be higher than the frequency of CMV-specific CD4+ T cells (compare figure 3 third row of panels and lower panels). Moreover, after SEB stimulation, a population of cells appears that is activated (i.e. CD69+) but does not produce IFN-γ. Naive T cells can not readily produce effector cytokines like IFN-γ upon first short term stimulation. Therefore, these cells presumably are naive T cells.

The effects of a single dose of rhIFN-γ on CMV- and SEB-specific CD4+ T cell reactivity in time is visualized in figure 4. In patients receiving placebo, CMV-specific and SEB-specific CD4+ T cell reactivity decreased over time from day 2 to day 4 after the surgical intervention. In contrast, in patients treated with rhIFN-γ, CMV-specific helper cell reactivity gradually increased (figure 4, left panel). Administration of rhIFN-γ significantly increased SEB-specific CD4+ T cell reactivity (figure 4, right panel). Moreover, the decrease of CMV- and SEB-specific CD4+ T cell reactivity over the two days after placebo treatment was significantly different from the increase in the rhIFN-γ treated patients (table 2).
Distribution of naive and memory CD4⁺ T cell subsets

Administration of rhIFN-γ is known to affect the distribution of peripheral blood leukocyte subsets. In all patients after the surgical procedure, a considerable leukocytosis was observed, consisting mainly of neutrophilic granulocytes. This leukocytosis gradually decreased in the study period in both the rhIFN-γ and the placebo treated groups. In both, the rhIFN-γ and the placebo treated patients, total lymphocyte counts did not change during the study period (not shown).

Effector cytokine producing CD4⁺ T cells are located exclusively in the memory population and therefore changes in subset distribution could affect the outcome of the above described functional assays (20,30). Composition of the circulating T cell subsets was evaluated in the study populations. Mean frequencies of CD45RA⁻ memory CD4⁺ T cells decreased in the rhIFN-γ treated patients, but not in placebo treated patients (figure 5). In contrast, the frequency of CD45RA⁺CD27⁺ naive CD4⁺ T cells significantly increased in the rhIFN-γ treated patients, but not in patients receiving placebo (not shown). Moreover, the increase of naive CD4⁺ T cell frequency in the rhIFN-γ treated patients over the first day after treatment was significantly different from the decrease in naive CD4⁺ T cell frequency in the
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Figure 5 Administration of rhIFN-γ in patients after surgery induces a relative decrease of CD45RA⁺ memory CD4⁺ T cells in peripheral blood:
Time (in days after administration of IFN-γ or placebo, X-axis) versus frequency of CD45RA⁺ memory cells within the CD4⁺ T cell population (percentage of CD4⁺ lymphocytes, Y-axis). Circles represent means of rhIFN-γ (closed circles) or placebo- (open circles) treated patients, error bars represent the standard error of the mean.

Discussion

The present study illustrates that the many known correlates of boosted immune reactivity by IFN-γ contribute to increased in vivo antigen specific immunity in a system of surgically induced immunosuppression. We show that administration of rhIFN-γ increases densities of CD4⁺ T cell receptor ligands on monocytes, and concomitantly prevents a loss of antigen specific CD4⁺ T cell reactivity.
Physiologically, IFN-γ is produced by T cells and natural killer (NK) cells. The IFN-γ receptor is ubiquitously expressed (31). Dimerization of IFN-γ-receptors induces phosphorylation and thereby activation of the tyrosine-kinases JAK1- and JAK2. These JAKs then phosphorylate the homodimerized IFN-γ receptor α-chains. This results in a docking site for STAT1α. Docked STAT1α is subsequently tyrosine-phosphorylated, dissociates from the IFN-γ receptor and forms homodimers. STAT1α homodimers translocate to the nucleus and bind to the gamma activated site (GAS, TTNCNNNAA) of promoter regions of many genes. Among the genes is CIITA, a transactivator responsible for IFN-γ mediated upregulation of MHC class II expression (32).

Proteolysis of endocytosed proteins for presentation in class II is mediated by lysosomal cathepsins. Expression of some of these proteins in macrophages is induced by IFN-γ stimulation. The invariant chain (essential for class II assembly and stabilization) and HLA-DM (essential for loading of peptides in HLA class II

<table>
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<tr>
<th>Parameter</th>
<th>intervention</th>
<th>n=</th>
<th>average change</th>
<th>p-value</th>
<th>n=</th>
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<td>placebo</td>
<td>6</td>
<td>-15.41</td>
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<td>Mean average changes of monocyte HLA class II expression and antigen specific CD4+ T cell immune reactivity.</td>
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1 Mean fluorescence intensity in arbitrary units
2 days after administration of IFN-γ or placebo
3 null hypothesis: average change after placebo = average change after rhIFN-γ, Mann-Whitney test
4 nt: not tested, difference between day0 and day1 in both groups was not statistically significant
ns: not statistically significantly different
molecules) genes are coordinately expressed with MHC class II molecules and therefore upregulated by IFN-γ. These mechanisms contribute to increased availability of T cell receptor ligands. We favor a model by which these cooperating mechanisms lead to a higher amount of triggered T cell receptors. Moreover, IFN-γ induces integrin expression on monocytes (15). This may ameliorate formation of the immunological synapse, leading to more efficient TCR triggering and thereby to increased T cell reactivity.

In a subgroup of our patients, expression of the co-stimulatory molecules CD80 and CD86 on monocytes was analyzed. CD86 expression tended to increase on monocytes of rhIFN-γ treated patients whereas it remained unchanged in placebo treated patients (not shown). Thus, lowering of triggering thresholds of individual T cells may have occurred due to increased co-stimulatory activity (27,33). In addition, CD4+ T cells may express functional IFN-γ receptors. Therefore, direct effects of IFN-γ on CD4+ T cells may have affected intrinsic activation thresholds of T cells.

Next to HLA class II regulation, IFN-γ exerts many additional regulatory functions. Gamma activated sites are found in the promotor regions of the interferon regulatory factors such as IRF-1, IRF-2. These interferon regulatory factors contain N-terminal binding domains recognizing interferon-stimulated response elements (ISRE’s) of enhancer regions like the 5’ enhancer region of MHC class I genes. IRF-1 binding to ISRE in MHC class I enhancer regions together with nearby NFκB binding, induces MHC class I gene expression. Similarly, cooperation of NF-κB and IRF-1 binding regulate β2 microglobulin expression, necessary for MHC class I induction (31). For peptide presentation in class I molecules, proteolysis of ubiquitinated proteins starts in the proteasome. Subunits of the proteasome as well as the proteasome activator PA28 are induced by IFN-γ. After degradation, peptides are transported into the endoplasmic reticulum by the transporter associated with antigen processing (TAP), which is induced by IFN-γ. Next, tapasin mediates peptide loading on MHC class I molecules. Tapasin expression is also regulated by IFN-γ (31). Although not studied here, these mechanisms may contribute to better CD8+ T cell functions in rhIFN-γ treated patients.

During the last decade, chemokines have been found to regulate adherence to endothelium and extravasation of immune cells to inflammatory sites.
Expression of many of these chemokines are influenced by IFN-γ signaling (17). Administration of rhIFN-γ caused a redistribution of CD4+ T cells. CD45RA+CD27+ naive CD4+ T cells are increased in the circulation, whereas memory helper cells tend to decrease in peripheral blood. Effector cytokine producing CD4+ T cells are located solely in the memory population. Therefore, the here found rescue from decreased antigen specific CD4+ T cell reactivity is very unlikely to be due to selectively remaining cells bearing CMV- or SEB-specific T cell receptors. Taken together, these data favor a model by which, in a situation of post operative immunosuppression, administration of rhIFN-γ upregulates TCR-ligands on antigen presenting cells and thereby increases T cell receptor triggering efficiency.

A considerable portion of patients after pylorus-preserving pancreaticoduodenectomy are reported to acquire any form of post operative infection (34). Since CD4+ T cells orchestrate many aspects of antigen specific immunity against micro organisms, like B cell responses and cytotoxic T cell responses, our findings suggest an ameliorating effect of rhIFN-γ administration on antigen specific immune defenses in patients after abdominal surgery.

Acknowledgements

F.N.J. van Diepen and R. Reijneke are thanked for excellent technical assistance, Jan Weel and technicians of the department of clinical virology for determining the patient’s serostatus. Tanox Pharma is thanked for providing the anti-B7.1 and anti-B7.2 monoclonal antibodies. R.J. Rentenaar is supported by a grant from the Dutch Kidney Foundation grant number 95-1455

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