Immune responsiveness in immunosuppressed patients

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CHAPTER 6: INTERFERON-GAMMA ADMINISTRATION AFTER ABDOMINAL SURGERY RESCUES ANTIGEN-SPECIFIC HELPER T CELL IMMUNE REACTIVITY

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Abstract

Antigen induced activation of T cells is determined by many factors. Among these factors are 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 co-stimulatory 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 (IFNγ) is a type II homodimeric cytokine with multiple immunostimulatory properties. Several studies have been performed to assess the effects of IFNγ treatment in patients in need of increased immune reactivity. However, until now, the effect of IFNγ on human antigen specific CD4^pos T cell reactivity after surgically induced immunosuppression has not been reported. Therefore, a comparative trial of recombinant human (rh) IFNγ 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 rhIFNγ rescued downmodulation of antigen specific CD4^pos T cell reactivity, concomitant with an upregulation of TCR-ligands on antigen presenting cells. Selected patients may benefit from the immunostimulatory properties of rhIFNγ 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<sup>pos</sup> T cells is diminished[7-9].

Administration of recombinant human IFNγ has been performed in severe burn patients and trauma patients. Although a decline of infection rate or death rate upon IFNγ 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 rhIFNγ does reduce the frequency and severity of infections in patients with chronic granulomatous disease[14]. Finally, after IFNγ treatment of patients suffering from disseminated non-tuberculous Mycobacterial infections, clinical improvements have been reported[15]. IFNγ exerts various effects on the immune system *in vivo*. First, IFNγ depresses peripheral blood leukocyte counts, presumably by redistribution of cells from the circulation to secondary lymphoid organs and solid tissues[16; 17]. Second, IFNγ increases Fcγ-receptor-1 (FcγRI) expression on neutrophilic granulocytes, thereby increasing the Fc mediated, but not complement mediated phagocytosis of bacteria[16; 18]. Third, IFNγ increases FcγRII and III- and integrin expression on monocytes and hydrogen peroxide production by monocytes[10; 16]. Fourth, it has been demonstrated that IFNγ augments levels of lipopolysaccharide-binding protein[16].

After surgical interventions and sepsis, IFNγ administration reverses depressed HLA class II expression on monocytes[19; 20]. Thereby, densities of TCR-ligands for CD4<sup>pos</sup> T cells are reestablished at high levels on cells that may differentiate into dendritic cells[21]. It has not been previously resolved whether these effects of IFNγ elevate antigen specific T cell immune reactivity in vivo in humans. Recently developed tools allow determination of frequency and phenotype of antigen specific CD4<sup>pos</sup> T cells from human peripheral blood[22-25]. These assays depend on antigen induced CD4<sup>pos</sup> T cell activation and production of cytokines, intracellular cytokine staining and flowcytometric analysis. We performed a comparative, placebo controlled study into the effects of rhIFNγ on antigen-specific immunity of patients after a pylorus-preserving pancreaticoduodenectomy. During a study period of two days after rhIFNγ administration, characteristics of antigen-specific CD4<sup>pos</sup> 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 rhIFNγ elevates antigen specific CD4<sup>pos</sup> T cell reactivity after
surgical trauma.

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<sup>pos</sup> lymphocytes in peripheral blood [22; 25] and therefore, CMV-seronegative patients were excluded from this study. Other exclusion criteria were: 1) jaundice at hospital admission (bilirubin levels exceeding 40 μmol/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. Blood was sampled for determination of CMV serostatus and routine biochemical and hematological measurements. Two days after surgery and just before administration of recombinant human (rh) IFNγ or placebo, blood was drawn for determination of monocytes, lymphocyte subsets and CMV- and SEB-specific CD4<sup>pos</sup> T cells. At 09.00 A.M., rhIFNγ (100 μg/m<sup>2</sup>, 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 rhIFNγ or saline, blood was drawn, again for determination of monocytes, lymphocyte subsets and CMV- and SEB-specific CD4<sup>pos</sup> 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.

Immunofluorescent staining 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 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 rhIFNγ 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>pos</sup>CD27<sup>pos</sup> cells within gated CD4<sup>pos</sup> lymphocytes were determined using Cellquest software (BD) and designated naive CD4<sup>pos</sup> T cells[26-28]. The CD45RA<sup>neg</sup>CD27<sup>pos</sup>CD4<sup>pos</sup> and CD45RA<sup>neg</sup>CD27<sup>neg</sup>CD4<sup>pos</sup> T cell frequencies were added and designated memory CD4<sup>pos</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>pos</sup> T cells**

CMV- and SEB-specific CD4<sup>pos</sup> T cell frequencies were determined according to the method described by Suni et al. [24]. 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 CD4pos 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 lysis 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-IFNy-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 CD69posIFNypos cells within the CD4pos lymphocyte gate were determined using Cellquest software (BD) and designated CMV-specific CD4pos T cell frequency in case of CMV stimulated cultures or SEB-specific CD4pos T cells in case of SEB stimulated cultures.

Statistical analysis
Baseline characteristics were analyzed using either the Mann-Whitney test (continuous numerical variables, or Fisher’s exact test (proportions). 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 (i.e. IFNγ treated versus placebo treated) were analyzed by the Mann-Whitney test. Tests were performed using SPSS 7.5.3 software (SPSS Inc., Chicago, Illinois, USA)

Results
Patients
From December 1998 until February 2000, 12 patients were included into this study. Six patients were assigned to rhIFNγ treatment and six patients received placebo, both at day 2 after surgery. Patients in both groups were comparable with respect to sex, (female/male ratio: 4/2 in placebo treated patients respectively 1/5 in IFNγ treated patients, p<0.05 Fishers exact
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test) median age at entry into the study (54.5, range 49-69 years in placebo treated patients respectively 64, range 48-77 years in IFNγ treated patients p<0.05 Mann-Whitney), and median duration of the surgical procedure (as measured by median duration of anaesthesia, 285, range 210-330 minutes in placebo treated respectively 367.5 range 155-425 minutes in IFNγ treated patients, p<0.05 Mann-Whitney).

Expression of HLA-class II on peripheral blood monocytes
Before treatment, HLA class II expression on monocytes was low. In accordance with previous reports, treatment with rhIFNγ dramatically increased expression of HLA class II on monocytes (figure 1a and b)[10; 11; 16; 19; 20].

Figure 1 Administration of recombinant human interferon-gamma but not placebo induces monocyte HLA class II expression
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 rhIFNγ and represent measurements obtained just before administration of rhIFNγ (day 0, left panel), 24 hours after administration of rhIFNγ (day 1, middle panel) and 48 hours after administration of rhIFNγ (day 2, right panel)
Time (in days after administration of IFNγ or placebo, X-axis) versus HLA-class II expression on monocytes (in mean fluorescence intensity (arbitrary units), Y-axis) of rhIFNγ- (closed circles) and placebo- (open circles) treated patients. Circles represent means, error bars represent the standard error of the mean.

In contrast, 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 1b). Thus, administration of rhIFNγ increases densities of potential T cell receptor-ligands on antigen presenting cells, whereas in placebo treated patients T cell receptor ligand-densities remain depressed during the study period.
CMV-specific and SEB-specific CD4<sup>pos</sup> T cell reactivity in peripheral blood is rescued by rhIFNγ treatment

Figure 2 shows the development of CMV- and SEB-specific CD4<sup>pos</sup> T cell frequencies from one representative patient during the study period. In cultures incubated either without antigen (not shown) or with control antigen, no IFNγ-producing cells are found (figure 2 upper left panel). Treatment with rhIFNγ did not increase the frequencies of cells positive for IFNγ staining after incubation without antigen (not shown) or with control antigen. Therefore, occupancy of IFNγ-receptors by rhIFNγ is not detectable by flow cytometry in peripheral blood derived CD4<sup>pos</sup> T cells at 24 hours or 48 hours after rhIFNγ administration in these patients. Moreover, plasma levels of IFNγ are back to normal 24 hours after rhIFNγ administration[17]. In cultures incubated with CMV antigen, a small but clearly distinguishable CD4<sup>pos</sup> T cell population was detectable consisting of activated (CD69<sup>pos</sup>), IFNγ producing cells (figure 2, second 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 the cultures with MHC class II reactive antibodies[22-24]. 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 IFNγ (compare figure 2 second row of panels and third row of panels). This increase in the frequency of antigen specific CD4<sup>pos</sup> T cells by CD28 is likely due to a lowering of the activation threshold for IFNγ production by cells that would not have been sufficiently triggered in the absence of additional costimulatory signals[22; 23; 29].

CD4<sup>pos</sup> 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 IFNγ. 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 [30; 31]. The lower panels of figure 2 show the frequency of SEB-reactive CD4<sup>pos</sup> T cells from one representative patient before administration of rhIFNγ. Generally, the frequencies of SEB-reactive CD4<sup>pos</sup> T cells was found to be higher than the frequency of CMV-specific CD4<sup>pos</sup> T cells (compare figure 2 third row of panels and lower panels). Moreover, after SEB stimulation, a population of cells appears that is activated (i.e. CD69<sup>pos</sup>) but does not produce IFNγ. Naive T cells do not readily produce IFNγ upon first short term stimulation. Therefore, these cells presumably are naive T cells. The effects of a single dose of rhIFNγ on CMV- and SEB-specific CD4<sup>pos</sup> T cell reactivity in time is visualized in figure 3. In patients receiving placebo, CMV-specific and
SEB-specific $\text{CD}4^{\text{pos}}$ T cell reactivity decreased over time from day 2 to day 4 after the surgical intervention. In contrast, in patients treated with rhIFN$\gamma$, mean CMV-specific helper cell reactivity gradually increased (figure 3, left panel). Administration of rhIFN$\gamma$ significantly increased SEB-specific $\text{CD}4^{\text{pos}}$ T cell reactivity (figure 3, right panel).

Moreover, the decrease of CMV- and SEB-specific $\text{CD}4^{\text{pos}}$ T cell reactivity over the two days after placebo treatment was significantly different from the increase in the rhIFN$\gamma$ treated
patients in the same study period (table I). Thus, administration of IFNγ after surgical intervention increases antigen specific CD4^{pos} T cell activation.

![Figure 3](image)

Figure 3 Surgically depressed antigen specific CD4^{pos} T cell reactivity is rescued by in vivo administration of rhIFNγ but not by placebo.

Time (in days after administration of IFNγ or placebo, X-axis) versus frequency of CMV-specific CD4^{pos} T cells (left panel, percentage of CD4^{pos} lymphocytes, Y-axis) or frequency of SEB-specific CD4^{pos} T cells (right panel, percentage of CD4^{pos} lymphocytes, Y-axis). Circles represent means of rhIFNγ (closed circles) or placebo-(open circles) treated patients, error bars represent the standard error of the mean.

Table I Between groups analyses: Mean average changes of monocyte HLA class II expression and antigen specific CD4^{pos} T cell immune reactivity. Comparison of placebo treated patients with IFNγ treated patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>intervention</th>
<th>day 1-0^{2}</th>
<th>p-value</th>
<th>day 2-0^{2}</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFI HLA class II on monocytes</td>
<td>placebo</td>
<td>6 -15.41</td>
<td>0.041</td>
<td>5 40.51</td>
<td>0.032</td>
</tr>
<tr>
<td>% CMV-specific within CD4^{pos} lymphocytes no CD28</td>
<td>placebo</td>
<td>5 0.26%</td>
<td>ns</td>
<td>5 -0.18%</td>
<td>ns</td>
</tr>
<tr>
<td>% CMV-specific within CD4^{pos} lymphocytes with CD28</td>
<td>rhIFNγ</td>
<td>3 0.32%</td>
<td>nt^{4}</td>
<td>3 0.29%</td>
<td>0.036</td>
</tr>
<tr>
<td>% SEB-specific within CD4^{pos} lymphocytes</td>
<td>placebo</td>
<td>6 0.03%</td>
<td>nt^{4}</td>
<td>6 -0.31%</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>rhIFNγ</td>
<td>6 0.24%</td>
<td>nt^{4}</td>
<td>6 0.93%</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>placebo</td>
<td>6 -0.89%</td>
<td>nt^{4}</td>
<td>6 -0.68%</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>rhIFNγ</td>
<td>6 1.40%</td>
<td>nt^{4}</td>
<td>6 1.67%</td>
<td>0.026</td>
</tr>
</tbody>
</table>

1 mean fluorescence intensity in arbitrary units
2 days after administration of IFNγ or placebo
3 null hypothesis: average change after placebo = average change after rhIFNγ, Mann-Whitney test
4 nt: not tested, difference between day0 and day1 in both groups was not statistically significant
ns: not statistically significantly different
**Interferon-gamma administration after abdominal surgery rescues antigen-specific helper T cell immune reactivity**

**Distribution of naive and memory CD4\textsuperscript{pos} T cell subsets**

Administration of rhIFN\textsubscript{g} 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\textsubscript{g} and the placebo treated groups. In both, the rhIFN\textsubscript{g} and the placebo treated patients, total lymphocyte counts did not change during the study period (not shown). CD4\textsuperscript{pos} T cells that produce IFN\textsubscript{g} are located exclusively in the CD45RA\textsuperscript{neg} memory population and therefore, changes in subset distribution could affect the outcome of the above described functional assay [22; 32]. The composition of the circulating T cell subsets was evaluated in the study populations. Mean frequencies of CD45RA\textsuperscript{neg} memory CD4\textsuperscript{pos} T cells decreased in the rhIFN\textsubscript{g} treated patients, but not in placebo treated patients (figure 4).

In contrast, the frequency of CD45RA\textsuperscript{pos}CD27\textsuperscript{pos} naive CD4\textsuperscript{pos} T cells significantly increased in the rhIFN\textsubscript{g} treated patients, but not in patients receiving placebo (not shown). The increase of naive CD4\textsuperscript{pos} T cell frequency in the rhIFN\textsubscript{g} treated patients over the first day after treatment was significantly different from the decrease in naïve CD4\textsuperscript{pos} T cell frequency in the placebo treated patients (p=0.009 Mann-Whitney). These results point to a mechanism by which rhIFN\textsubscript{g} treatment causes a specific redistribution of memory CD4\textsuperscript{pos} T cells. Thus, administration of rhIFN\textsubscript{g} spares CMV-specific CD4\textsuperscript{pos} T cell reactivity and increases SEB-specific CD4\textsuperscript{pos} T cell reactivity in peripheral blood, whereas at the same time, the frequency of all memory CD4\textsuperscript{pos} T cells is reduced.

![Graph showing change in CD4^pos T cell subsets over time](image)

Figure 4: Administration of recombinant human interferon gamma in patients after pylorus-preserving pancreaticoduodenectomy induces a relative decrease of CD45RA\textsuperscript{neg} memory CD4\textsuperscript{pos} T cells in peripheral blood. Time (in days after administration of IFN\textsubscript{g} or placebo, X-axis) versus frequency of CD45RA\textsuperscript{neg} memory cells within the CD4\textsuperscript{pos} T cell population (percentage of CD4\textsuperscript{pos} lymphocytes, Y-axis). Circles represent means of rhIFN\textsubscript{g}- (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 IFNγ contribute to increased ex vivo antigen specific immunity in a system of surgically induced immunosuppression. We show that administration of rhIFNγ increases densities of CD4^{pos} T cell receptor ligands on monocytes i.e. HLA class II molecules, and concomitantly prevents a loss of antigen specific CD4^{pos} T cell reactivity. Monocytes from patients after abdominal surgery have similarities with the same cells analyzed in septic patients. In these patients, so called immunoparalysis occurs resulting in monocytic deactivation with decreased HLA class II expression and lowered LPS-induced TNFα production. Notably, the lowered TNFα production as well as the decreased HLA-class II expression of "immunoparalysis" in vivo were reversible by treatment with recombinant human IFNγ and, associated with surviving the septic episode[20; 33]. We have shown that after abdominal surgery, the decreased HLA-DR expression could be reversed by IFNγ treatment (de Metz et al. submitted for publication). Pre-incubation of monocytes with TNFα in vitro improves their capacity to induce antigen specific T cell activation[23]. Therefore, in our IFNγ treated patients, increased TNFα availability may have stimulated antigen presentation function of monocytes.

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 IFNγ stimulation. Next to this, IFNγ upregulates CIITA, a transactivator essential for MHC class II expression[34]. The invariant chain (essential for class II assembly and stabilization) and HLA-DM (essential for loading of peptides in HLA class II molecules) genes are coordinately expressed with MHC class II molecules and therefore upregulated by IFNγ. Therefore, these combined effects of IFNγ on the expression of proteolytic enzymes and on components of the MHC class II complex may contribute to increased availability of T cell receptor ligands. In addition, IFNγ induces integrin expression on monocytes[16]. 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 rhIFNγ 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[29; 35]. In addition, CD4^{pos} T cells may express functional IFNγ receptors. Therefore, direct effects of IFNγ on CD4^{pos} T cells may have affected intrinsic activation thresholds of T cells.
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 IFNγ signaling[18]. In the present study administration of rhIFNγ caused a redistribution of CD4pos T cells. CD45RAposCD27pos naive CD4pos T cells were increased in the circulation, whereas memory CD4pos T cells decreased in peripheral blood. CD4pos T cells that produce IFNγ upon stimulation are located solely in the memory population[32]. Therefore, the rescue of CMV-specific CD4pos T cell reactivity and increase in SEB-specific CD4pos T cell reactivity can not be explained by an increased frequency of circulating T cells bearing CMV- or SEB-specific T cell receptors, but rather reflects improved T cell activation.

Taken together, these data favor a model by which, in a situation of post-operative immunosuppression, administration of rhIFNγ upregulates TCR-ligands on antigen presenting cells. Next, IFNγ induces expression of adhesion- and costimulatory molecules on monocytes and activating cytokines by monocytes and thereby increases efficiency of T cell receptor triggering and lowers net thresholds for CD4pos T cell activation. Since CD4pos T cells cooperate with immune cells like B cells and CD8pos T cells, this increased CD4pos T cell reactivity may be important in regulation of antigen specific immunity. A considerable portion of patients after pylorus-preserving pancreaticoduodenectomy are reported to acquire any form of post operative infection[36]. Restoration of immune function in these patients by IFNγ administration may be beneficial.

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Reference List


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Chapter 6


