

Review

Perioperative IFN- α to avoid surgically induced immune suppression in colorectal cancer patients

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Summary. Surgical treatment of colorectal cancer is associated with postoperative immunosuppression, which might facilitate dissemination of tumor cells and outgrowth of minimal residual disease/(micro) metastases. Minimal residual disease has been shown to be of prognostic relevance in colorectal cancer. Therefore, stimulation of (anti-tumor) immune responses may be beneficial in the prevention of metastases formation. Important anti-tumor effector cells, which serve this function, are natural killer (NK) cells, CD8+ lymphocytes (CTL), dendritic cells (DC) and macrophages. In this review the immunomodulating properties of IFN- α are discussed, with a particular focus on perioperative stimulation of immune function in cancer patients.

IFN- α is known to enhance innate immune functions such as stimulation of NK cells, transition from innate to adaptive responses (activation of DC) and regulating of CD8+ CTL activity and memory. Moreover, it exerts direct antitumor effects by regulating apoptosis and cell cycle. In several clinical trials, perioperative administration of IFN- α has indeed been shown to improve T cell responsiveness, prevent impairment of NK cell cytotoxicity and increase expression of activation markers on NK, T and NKT cells. In a clinical pilot study we showed in colorectal cancer patients that received perioperative IFN- α enhanced activation markers on T cells and NK cells, combined with better-preserved T cell function as indicated by phytohemagglutinin skin tests. In the liver of these patients significantly more CD8+ T cells were found. In conclusion, IFN- α provides an effective adjuvant in several forms of cancer and improves several postoperative immune functions in perioperative administration. However, larger clinical trials are

necessary to investigate effects on disease-free and overall survival.

Key words: Interferon α (IFN- α), Immune function, Surgery. Colorectal cancer

Introduction

Surgical resection is the mainstay in the initial treatment of colorectal cancer. However, major abdominal surgery is associated with transient postoperative immunosuppression, which might facilitate dissemination of tumor cells and outgrowth of micro metastases (Sietses et al., 1999; Mels et al., 2001). Animal studies have shown increased tumor outgrowth following major surgery, which may be explained by enhanced adhesion of isolated tumor cells, stimulated growth of micrometastases and (facilitated by) surgery-induced immunosuppression (Bouvy et al., 1997; Sietses et al., 1999; Raa et al., 2005). The magnitude and duration of surgical trauma correlates to the degree of immunosuppression (Sietses et al., 1999, 2000; Gupta and Watson, 2001). Particularly important with respect to immunosuppression are the first postoperative days, as free circulating and intraperitoneal tumor cells (and minimal residual disease) have been demonstrated during and after the operation in colorectal cancer patients (Schott et al., 1998; Sales et al., 1999; Bosch et al., 2003). Moreover, the presence of disseminated cancer cells in peripheral blood and peritoneal cavity of these patients has been shown to serve as prognostic factor (Bosch et al., 2003).

In order to eliminate minimal residual disease and disseminated cancer cells, improvement of immune function in the postoperative phase might be a particularly appealing aim in patients who undergo surgical resection of a primary carcinoma. Moreover, improved anti-tumor immune responses may even have

advantageous implications for long-term prognosis of cancer patients. Important effector cells that serve this anti-tumor immune response are natural killer (NK cells), cytotoxic T lymphocytes (CTL), macrophages and dendritic cells (DC). As interferon-alpha (IFN- α) activates these effector cells via both innate and adaptive immunity it might be an eligible candidate to provide the enhancement of anti-tumor immune responses. Stimulation of NK cell cytotoxic function, antigen-dependent cellular cytotoxicity of macrophages as well as stimulation of DC function are some of its effects on innate immunity. In addition, IFN- α can exert direct anti-tumor effects by modulating tumor cell proliferation, apoptosis and cell cycle (Belardelli et al., 2002; Belardelli and Ferrantini, 2002).

Immunotherapy with IFN- α has been shown to improve prognosis in several malignancies such as melanoma, renal cell cancer, Kaposi's sarcoma and various haematological malignancies including chronic myeloid leukemia, hairy cell leukemia and multiple myeloma (Tagliaferri et al., 2005). In spite of its acknowledged anti-tumor effectiveness, which can range from tumor regression to symptomatic relief in end-stage cancer patients, the precise mechanisms of action of IFN- α in clinical treatments have not been fully elucidated. In this review we summarize data on the effects of IFN- α as immuno adjuvant in surgical treatment of a primary tumor, focussing on perioperative applications in colorectal cancer patients.

IFN- α

The type I interferon family consists of several subtypes including IFN- α , β , and ω , that use the same heterodimeric receptor, generally expressed on most cell types. Likewise, type I interferons exert comparable biological properties, ranging from defense against viral infection to anti-neoplastic effects (Belardelli et al., 2002). IFN- α is constitutively expressed by many cell types, including T cells, NK cells, monocytes/macrophages, dendritic cells (DCs) and fibroblasts (Brassard et al., 2002). Viral or bacterial infection induces high levels of IFN- α production by infected cells, resulting in activation of an innate defense, which comprises amongst others NK cells and macrophages. As it strongly inhibits viral replication, recombinant IFN- α was initially developed as an antiviral agent and is currently widely used in the treatment of hepatitis C. Additionally, its efficacy in immunotherapy for several types of haematological as well as solid malignancies has been shown in the clinic (Tagliaferri et al., 2005).

Stimulation of innate immune responses

IFN- α is a potent stimulator of innate immune responses, most notably exemplified by the direct enhancement of cytotoxicity mediated by NK cells that play an important role in initial (innate) responses against virally infected cells (Brassard et al., 2002; Liang

et al., 2003). Furthermore, NK cells have important anti-tumor effector functions. Enhancement of NK cell cytotoxicity by IFN- α has been shown *in vitro* as well as *in vivo* and is mediated through up-regulated gene expression of cytolytic effectors such as Fas-L, perforin and IFN- α (Biron, 2001; Liang et al., 2003; Kamath et al., 2005). Additionally, IFN- α stimulates proliferation and trafficking of NK cells (Salazar-Mather et al., 1996; Biron, 2001). Although less well characterised than effects on NK cells, cytotoxic activation of macrophages has also been reported (Belardelli and Ferrandini, 2002), as antigen-dependent cellular cytotoxicity of macrophages against mouse thymoma R1 target cells was shown to be increased upon stimulation with IFN- α (Ralph et al., 1988).

IFN- α (linking innate) and adaptive immune responses

Besides effects on innate immune cells, IFN- α has a strong regulatory effect on immune cells that link innate and adaptive responses. Dendritic cells (DCs), located in peripheral tissues play an essential role in the transition of innate to adaptive immune responses, as they are able to present pathogenic and tumor antigens. Following antigen uptake, DCs undergo maturation and migrate toward the local draining lymph node. Here, the (tumor-associated) antigen is processed and presented to CD8+ or CD4+ T-cells by respectively MHC class I or MHC class II molecules, which are expressed on the surface of DCs (Delves and Roitt, 2000). IFN- α provides a signal for DC maturation and activation by several mechanisms, including up-regulation of co-stimulatory molecules that promote interaction with T cells. (Belardelli and Ferrantini, 2002). In turn, appropriately activated DCs are able to secrete high levels of IFN- α themselves, which is proposed to act in an autocrine manner (Montoya et al., 2002). It was shown that bone marrow-derived DCs from IFN- α receptor deficient mice had lower expression of costimulatory cell surface molecules and were less capable of stimulating CD4+ and CD8+ cells than DCs derived from control mice (Montoya et al., 2002). Thus, IFN- α can provide a signal for DC differentiation and activation, resulting in effective presentation of tumor antigens.

Moreover, IFN- α has several major regulating and facilitating effects on adaptive immunity, such as activation, proliferation and long-term survival of CD8+ cytotoxic T-cells (CTLs) and memory T-cells (Marrack et al., 1999; Brassard et al., 2002). Additionally, a role for IFN- α in the promotion of Th1/Th2 response towards the Th1 subset has been proposed (Belardelli and Gresser, 1996; Tough et al., 1996; Wenner et al., 1996; Brassard et al., 2002). In mice, IFN- α has been shown to direct T cell differentiation by a decrease in IL-4 gene expression and to enhance production of antibodies (IgG2 α) (Finkelman et al., 1991).

Taken together, these data emphasize the wide range of complex immune stimulating effects of IFN- α on both innate and adaptive immune responses that could

potentially result in effective anti-tumor responses.

IFN- α as immunotherapeutic in cancer

Direct effects of IFN- α

IFN- α has been shown to directly inhibit proliferation of a variety of cancer cell types (Balkwill and Oliver, 1977), as it activates several genes encoding pro-apoptotic proteins and repression of anti-apoptotic genes (Matarrese et al., 2002; Clemens, 2003). Transcriptional activation of CD95 (Fas/Apo-1), a member of the tumor necrosis factor (TNF) receptor family and a potent inducer of apoptosis has been described as a mechanistic pathway. CD95 activates the caspases cascade subsequently leading to DNA fragmentation and apoptosis. Recently, activation of the p53 tumor suppressor gene was also shown to be directly induced by IFN- α and to play a pivotal role in the IFN- α induced apoptotic pathway through activation of CD95 (Porta et al., 2005). Another direct anti-tumor mechanism of IFN- α comprises the upregulation of enzymes that facilitate the retardation of the cell cycle progression, such as cyclin-dependent kinase inhibitors (Tagliaferri et al., 2005).

Alternatively, IFN- α up-regulates especially class I, but also class II major histocompatibility complex (MHC) expression on tumor cells resulting in improved antigen recognition (through immunosurveillance), presentation and subsequent promotion of CD8+ T-cell responses (Palmer et al., 2000; Biron, 2001).

IFN- α in cancer models

In transplantation models in athymic mice, which lack T cells, antibodies directed against IFN- α resulted in enhanced tumor outgrowth with less host cell infiltration and increased incidence of tumor invasion and metastases. These observations implied that IFN- α induced a host anti-tumor reaction that was probably not T cell mediated (athymic mice) (Reid et al., 1981; Gresser et al., 1983; Gresser and Belardelli, 2002). Treatment with anti-IFN- α antibodies in immunocompetent mice also resulted in more rapid tumor growth, even when IFN- α resistant tumor cell lines were used, indicating that IFN- α acted through host immune mechanisms and not via direct effects on tumor cells (Gresser et al., 1983).

Alternatively, several studies using genetically modified tumor cells that expressed IFN- α have revealed the importance of the host's immune system in the generation of an IFN- α induced immune response. Highly metastatic Friend leukemia cells (FLC) that were transfected to produce IFN- α failed to develop into tumors in normal mice, but were highly tumorigenic in immunosuppressed nude mice, indicating the importance of T cells in this immune response (Ferrantini et al., 1993). Moreover, administration of IFN-producing FLC to normal mice resulted in a long lasting immune

resistance to subsequent rechallenge with highly metastatic parental FLC (Gabriele et al., 1995). Contralateral injection of IFN- α producing FLC in mice that had already established subcutaneous tumors resulted in significant growth inhibition of the existing tumor which indicates that induction of a potent anti-tumor immune response (T-cell mediated) is induced by IFN- α (Ferrantini et al., 1993). Furthermore, highly metastatic mouse mammary adenocarcinoma cells (TS/A-pc), which were transfected with the mouse IFN- α gene were efficiently rejected in BALB/c mice, whereas injection of parental TS/A-pc or control cells transfected with the empty vector led to development of large subcutaneous tumors (Ferrantini et al., 1994). Additionally, the expression of IFN- α by transfected TS/A-pc tumor cells resulted in a significant inhibition of metastases formation. Histologic examination showed that rejection of tumors was mainly attributed to activity of CD8+ T lymphocytes and partially to polymorphonuclear cells (PMNs) (Ferrantini et al., 1994). IFN- α gene transfection of B16 melanoma cells resulted in less tumor growth than control cells in syngeneic mice and complete rejection in allogeneic mice (Kaido et al., 1995).

IFN- α/β therapy increased survival in DBA/2 mice that were immunized with highly malignant Esb lymphoma cells and subsequently rechallenged, suggesting cooperation between IFN- α therapy and the immune system. Consequently, adoptive transfer of Esb spleen cells from immunized animals together with IFN- α/β inhibited tumor development in immunocompetent naive mice. Interestingly, the protective effect of immunized ESb splenocytes in IFN- α/β treated mice was eliminated by depletion of CD8+ T cells (but not CD4+ T cells or B lymphocytes) prior to transfer, indicating the importance the CD8+ T cell population in IFN- α induced anti tumor immune response (Kaido et al., 1994). Thus, transduction of the IFN- α gene into different mouse tumor systems resulted in host-mediated and long-term tumor specific memory, suggesting complicated involvement of many immune cells and pathways.

Clinical trials

The promising finding that IFN- α exhibited both direct and a wide range of host-mediated effects in many *in vivo* models prompted initiation of clinical trials applying IFN- α treatment in various malignancies, (combination) regimens and types of administration. Recombinant IFN- α -2a (Roferon) and the closely related IFN- α -2b (Intron-A) are the most used and closely related subclasses of IFN- α . The addition of PolyEthylene Glycol (pegylation; PEG FN- α) to manufactured FN- α greatly reduces the elimination rate allowing maintenance of an extended steady state level of the drug, which lessens the need for frequent injections.

The precise anti-tumor mechanisms of IFN- α have

not completely been characterised in humans. Adjuvant treatment with IFN- α in high-risk resected melanoma patients resulted in enhanced cytotoxic NK cell and T cell function *ex vivo* compared to the observation group (Kirkwood et al., 2002). Additionally, IFN- α was shown to enhance anti-melanoma cytotoxic T lymphocyte (CTL) generation from peripheral blood lymphocytes that had been stimulated with irradiated primary melanoma cultures (Palmer et al., 2000). Hakansson et al. evaluated the histology of resected metastatic lesions in melanoma patients that had been treated with IFN- α for 1-3 weeks. Comparison between non-treated and increasing durations of IFN- α therapy indicated a positive trend for regressive changes in metastatic lesions in treated patients. These regressive alterations were accompanied by a significant recruitment of CD4+ cells close to tumor cells, while there was a weak effect on tumor infiltration of CD8+ cells (Hakansson et al., 1998). Importantly, treatment of melanoma patients with a high risk of recurrence after resection with high-dose IFN- α -2b resulted in improved relapse-free and overall survival (Kirkwood et al., 1996; Kirkwood et al., 2001).

Subcutaneous treatment with IFN- α 2b in patients with advanced renal cell carcinoma was evaluated for its immunorestorative properties and correlation with clinical response (Kosmidis et al., 1992). IFN- α 2b treatment resulted in a significant potentiation of the deficient cellular immune response (especially CTL function) in renal cell carcinoma patients who clinically responded to IFN- α therapy. Patients who did not respond to IFN- α therapy showed no change in immune status (Kosmidis et al., 1992).

Combinations of IFN- α treatment with other bioreagents or chemotherapy have been investigated with varying success. The addition of interleukin-2 (IL-2) has shown promising results in patients with advanced renal cell cancer (Rogers et al., 2000; Atzpodien et al., 2004). Since IFN- α was reported to augment the activity of 5-fluorouracil (5-FU) in addition to its enhancing effects on cellular immune responses, it has extensively been studied as adjuvant in chemo-immunotherapy in patients with (advanced) colorectal cancer (Nichols et al., 1994). However, the addition of IFN- α to 5-FU (or to the combination 5-FU and leucovorin) in large prospective randomized clinical trials did not provide any clinical benefit (Corfu-A Study Group, 1995; Hausmaninger et al., 1999).

Taken together, the adjuvant treatment of IFN- α has been shown to stimulate particularly NK and CTL function in (small) clinical trials in which mainly advanced or high-risk cancer patients participated.

IFN- α in the perioperative oncological setting

The development of local peritoneal recurrences may be facilitated by tumor cell spilling into the peritoneal cavity during resection surgery (Schott et al., 1998). Furthermore, major surgery, such as resection of primary colorectal cancer, is associated with transient

depression of immune functions within 2-4 hours after surgery, sustaining until approximately 5-10 days after surgery. Thus, surgical resection of a primary tumor accompanied by transient immune suppression in the postoperative phase may, paradoxically, create permissive circumstances for tumor cells to disseminate and grow.

Post surgical immune suppression mainly comprises cellular defects, affecting T cells, monocytes/macrophages, B cells and PMNs (Faist et al., 1996; Gupta and Watson, 2001). In addition, NK cell numbers and cytotoxicity were also suppressed following major surgery in cancer patients (Leung et al., 2003). Therefore, reduction of postoperative immune suppression by stimulating (anti-tumor) immune responses may be beneficial in the prevention of tumor dissemination. Since IFN- α has many effects on the abovementioned immune parameters including stimulation of T-cells, NK cells, DCs and monocytes, it is an attractive candidate for perioperative application.

In patients with advanced cancer undergoing surgery, Houvenaeghel et al. showed a non-significant decrease in NK cell activity following postoperative treatment with IFN- α -2a compared to pretreatment values of the same patients (Houvenaeghel et al., 1997). Sedman et al. randomly allocated gastric and colorectal cancer patients preoperatively to a 1-week course of subcutaneous recombinant human IFN- α -2a (r-hu IFN- α -2a) or to a control group. Cytotoxicity of NK cells from control patients was greatly reduced immediately after surgery in an *in vitro* chromium release cytotoxicity assay. Importantly, NK cell cytotoxicity in r-huIFN- α -2a treated patients did not decline in the postoperative period and levels were significantly higher than those observed in the control group on postoperative day 1, 3 and 5 (Sedman et al., 1988). However, treatment with r-huIFN- α did not prevent postoperative suppression of IL-2 production or lymphokine activated killer cell (a peripheral mononuclear blood leukocyte cultured in the presence of IL-2) cytotoxicity (Sedman et al., 1988). In another randomized clinical trial, colorectal cancer patients received either recombinant (r) IL-2, a combination of rIL-2 with IFN- α or control vehicle perioperatively (Nichols et al., 1993). NK cell cytotoxicity was augmented in the combined rIL-2 and IFN- α group on the first postoperative day, compared to both the rIL-2 alone and control group, which showed a decline in functionality (Nichols et al., 1993).

In a clinical pilot study, we investigated the effect of perioperative treatment with IFN- α on postoperative immunosuppression and on liver immunology in patients operated on for colorectal cancer, since the liver is the most important site for metastasis formation in colorectal cancer. Patients were operated on day 0 through median laparotomy with partial colectomy and received daily subcutaneous injections with 3.0 x 10⁶ IU IFN- α -2b (Intron A[®]) or 1 ml of saline from 3 days before until 4 days after surgery. A total of 9 patients

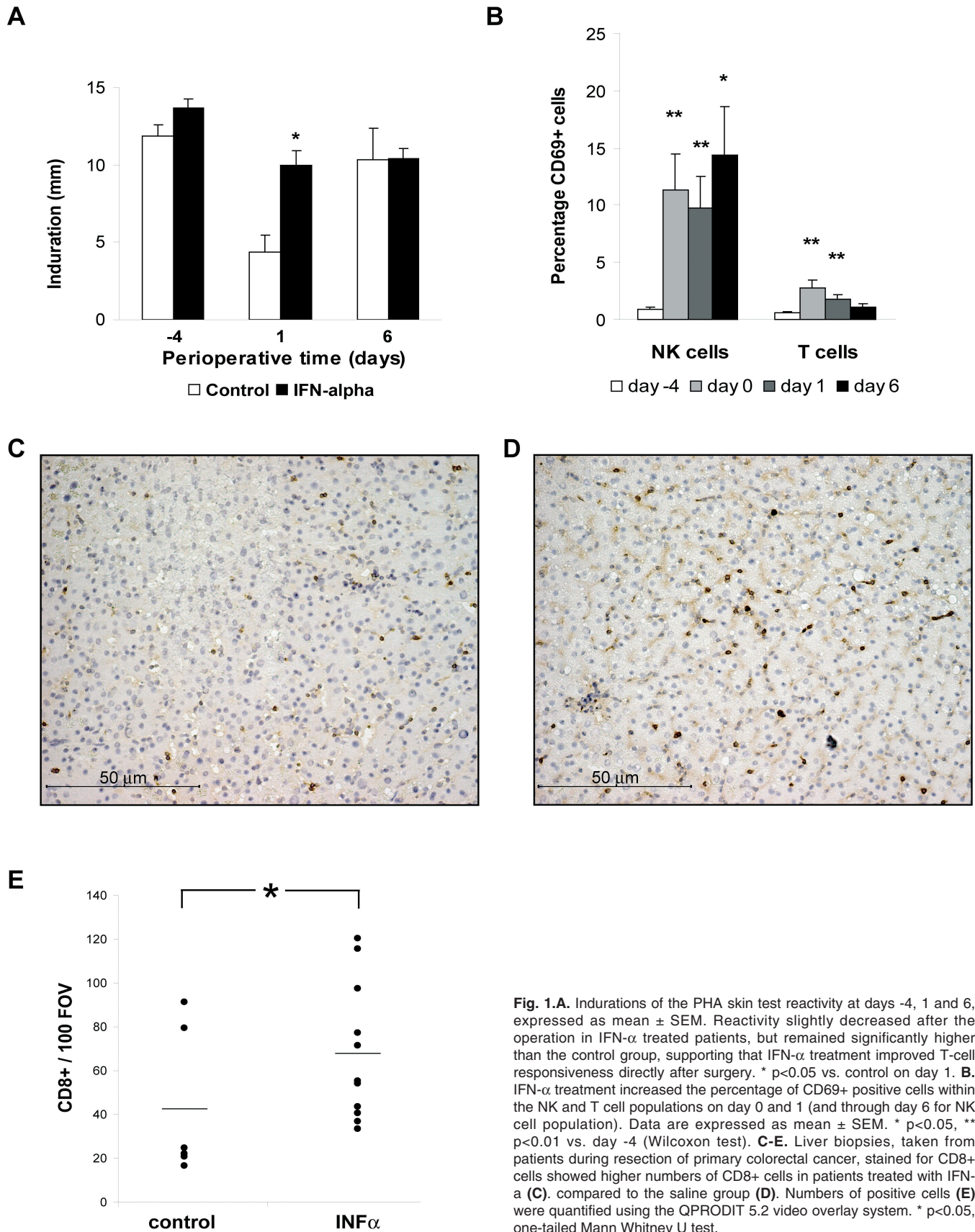


Fig. 1. **A.** Indurations of the PHA skin test reactivity at days -4, 1 and 6, expressed as mean $\pm$ SEM. Reactivity slightly decreased after the operation in IFN- α treated patients, but remained significantly higher than the control group, supporting that IFN- α treatment improved T-cell responsiveness directly after surgery. * $p < 0.05$ vs. control on day 1. **B.** IFN- α treatment increased the percentage of CD69+ positive cells within the NK and T cell populations on day 0 and 1 (and through day 6 for NK cell population). Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ vs. day -4 (Wilcoxon test). **C-E.** Liver biopsies, taken from patients during resection of primary colorectal cancer, stained for CD8+ cells showed higher numbers of CD8+ cells in patients treated with IFN- α (**C**), compared to the saline group (**D**). Numbers of positive cells (**E**) were quantified using the QPRODIT 5.2 video overlay system. * $p < 0.05$, one-tailed Mann Whitney U test.

were included in the IFN- α group and compared to patients that received GM-CSF or control treatment (Mels et al., 2001). Responsiveness to the mitogen phytohemagglutinin (PHA) was measured in a delayed type hypersensitivity skin test (size of induration as postoperative indicator of T cell function) (Mels et al., 2001). Indurations before surgery were not different between the IFN-IFN- α , GM-CSF and control groups. Although a slight decrease was observed on day 1, indurations in the IFN-IFN- α group were significantly higher than in the control group (Fig. 1A). Moreover, none of the IFN- α treated patients showed anergy (induration below 5 μ m) on day 1, while 6 out of 8 patients from the control group were anergic, supporting that IFN- α treatment improved T-cell responsiveness directly after surgery. In order to investigate activation of lymphoid cells, CD69 expression, which is an early activation marker, was measured on both NK cells and T lymphocytes. IFN- α treatment increased the percentage of CD69+ NK cells on day 0, +1 and day +6. Furthermore, CD69 expression was increased on T cells on day 0, which sustained on day +1 (Fig. 1B). Upregulation of the co-stimulatory molecule CD69 on immature NK cells has been linked to enhanced cytotoxic function and IFN- α production (Ziegler et al., 1994; Jewett and Bonavida, 1995; Clausen et al., 2003). These observations, which are in accordance with Sedman et al. support that the activated state of these cells persisted even after surgical trauma, thereby possibly improving cytotoxic capacities in the postoperative period.

In liver biopsies taken from patients during resection of primary colorectal cancer, numbers of CD3+ as well as CD8+ cells were significantly higher in patients treated with IFN- α compared to the saline group ($p < 0.05$, one-tailed Mann-Whitney U test) (Fig. 1C-E). The number of MHC class II expressing cells was however not altered, presumably because the number of CD68+ cells (Kupffer cells), which represent the most prominent populations in the liver, did not differ between IFN- α treated and control patients (data not shown). Taken together, this study showed that IFN- α minimized postoperative suppression of PHA skin test reactivity, which is indicative of a reduced impact of surgical trauma on immune status. Importantly, more activated T cells were present after IFN- α treatment. However, larger clinical trials are necessary to investigate effects on recurrence-free and overall survival.

Concluding remarks

IFN- α can exhibit direct anti-tumor effects and furthermore plays an important role in enhancing innate and adaptive immune responses, including stimulation of NK cells, transition from innate to adaptive responses and regulation of CD8+ CTL activity and memory responses. As responsiveness of immune cells is transiently impaired following major surgery, which may

have important consequences for facilitating outgrowth of (micro)metastases / minimal residual disease, IFN- α might minimize this transient immunosuppression and as such may be beneficial in the prevention of tumor dissemination. In small clinical trials, adjuvant treatment with IFN- α has been shown to stimulate particularly NK and CTL function. Perioperative administration of IFN- α has been shown to improve T cell responsiveness, prevent impairment of NK cell cytotoxicity and increase expression of activation markers on NK and CD8+ T cells in several clinical trials. In conclusion, IFN- α provides an effective adjuvant in several forms of cancer and improves several postoperative immune functions in perioperative administration.

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