**Interleukin-10 counteracts T-helper type 1 responses in B-cell lymphoma and is a target for tumor immunotherapy**

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**Highlights:**

* Th1 cells in a lymphoma microenvironment are converted to Tr1 cells
* Tr1 cells in lymphoma coexpress IFN- and IL-10 and upregulate PD-1
* IL-10 drives Tr1 induction and PD-1 upregulation
* IL-10 neutralization can be used for targeted therapy of lymphoma

**Abstract**

To establish strategies for immunotherapy of B-cell lymphoma, it is mandatory to gain deeper insights into the mechanisms of tumor immune escape. In a mouse model of endogenously arising lymphoma, we investigated the impact of IL-10 on the regulation of antitumor responses. Despite progressive functional impairment of NK cells and lack of IFN- in the tumor milieu, we found an augmented fraction of T helper type 1 (Th1) cells, which continued to express IFN- but also upregulated IL-10 during disease development. Using a lymphoma microenvironment *in vitro*, we showed that Th1 cells were converted to Foxp3-negative T regulatory type 1 (Tr1) cells, which coexpressed IFN- and IL-10 and upregulated PD-1. This differentiation required pre-existing IL-10, which was primarily provided by malignant B cells and dendritic cells. IFN- only declined in cells with the uppermost PD-1 levels. Importantly, antibody-mediated IL-10 ablation *in vivo* improved effector cell functions and significantly suppressed tumor development. While the contribution of IL-10 to cancer immune escape has been controversially discussed in the past, we show that IL-10 suppresses ongoing, potentially protective immune responses in lymphoma and might be a target for immunotherapy.

**Keywords:** Th1 cells, Tr1 cells, lymphoma, c-MYC, tumor escape, IL-10

**1. Introduction**

It is commonly accepted that T lymphocytes recognizing tumor-derived antigenic (Ag) peptides may be able to eliminate cancer cells. However, potentially protective immune effector cells frequently become functionally compromised in the tumor microenvironment, for example because of anergization or exhaustion (1). A proinflammatory microenvironment, hence the production of cytokines like interferon- (IFN-) and tumor necrosis factor (TNF), is necessary for the induction of Ag-specific CD4+ T cells of the Th1 subtype, which express IFN- on their part and endorse cytotoxic antitumor CD8+ T-cell responses, while Th2 responses rather promote tumor growth (2-6). Since natural killer (NK) cells, which are a major source of the Th1-inducing cytokine IFN- (7), become paralyzed following long-lasting activation, the tumor milieu may be characterized by a lack of IFN- (8, 9), which suggests a bias against Th1 differentiation.

To limit overshooting immune responses against pathogens and to prevent autoaggressive disorders, the immune system has evolved a finely tuned balance of stimulatory and counterregulatory mechanisms. A pivotal component of the inhibitory arm is the antiinflammatory cytokine interleukin-10 (IL-10), which decreases IFN- expression in Th1 cells (review in 10). The role of this cytokine for protecting from self damage is evidenced by the association of deficient IL-10 function with development of colitis (11-13). IL-10 expression can be induced in a huge plethora of immune effector cells, such as Th1, Th2, Th17 and FoxP3+ regulatory T (Treg) cells as well as in dendritic cells (DC), macrophages or B cells (10).

In light of its ability to counteract proinflammatory Th1 responses, it is not surprising that IL-10 is able to confer tumor immune escape (14-17). However, IL-10 can also play an opposite role being capable of fostering immune responses and increasing cytotoxic T-cell responses in murine or human tumors (18-21). These differential effects might depend on the tumor entity and the cellular networks involved.

In B-cell lymphoma, little is known on the effects of IL-10. Using mice that develop spontaneous lymphoma due to the presence of a *MYC* transgene under the control of the immunoglobulin  enhancer (22), we identified several mechanisms of immune escape in this disorder, for example progressive functional impairment of NK cells (8, 23), alteration of DCs (24) or immunosuppressive activity of Treg cells recognizing defined tumor-derived Ags (25). Specifically, increased levels of IL-10 were detected, which was primarily derived from the malignant cells (24). However, the significance of IL-10 for regulating potential antitumor responses remained elusive.

Given the close interplay between the innate and adaptive immune system, which eventually links NK-cell activation to the induction of antitumor T-cell responses of the Th1/Tc1 type (26, 27), the progressive loss of IFN- secretion by NK cells in B-cell lymphoma was expected to impede or reverse Th1 polarization. Here we show that, paradoxically, the fraction of IFN--producing intratumoral CD4+ T cells still increases even in late tumor stages. However, the IFN--expressing T cells are exhausted and upregulate IL-10 expression on their part. We provide evidence that IL-10 has an immunosuppressive function in the lymphoma model and thus can serve as a target for tumor immunotherapy.

**2. Materials and Methods**

**2.1. Animal experiments**

All animal experiments were approved by the competent authority and carried out in accordance with the EU Directive 2010/63/EU. Wildtype (wt) C57BL/6 and λ-MYC C57BL/6 mice (22) were bred in our animal facility, IFN-γ reporter with endogenous polyA tail (Great) mice (C57BL/6 background) were kindly provided by Dirk Baumjohann (Ludwig-Maximilians-Universität München). In these animals, an IRES element and a YFP sequence are inserted downstream of the stop codon of IFN-γ. All mice were housed under specified pathogen-free conditions. As soon as λ-MYC mice developed visible tumors, they were euthanized and the spleens were dissected for further analyses. The sex of the animals had no impact on the results.

For specific neutralization of IL-10, λ-MYC mice were injected intraperitoneally (i.p.) with 100 µg purified anti-IL-10 antibody (JES5-16E3; BioLegend, San Diego, USA) at day 55, 65, 75 and 85 after birth. Untreated λ-MYC mice served as a control.

For induction of specific Th1 cells, wt or Great mice were injected i.p. with 10 nmol CpG oligonucleotide 1668 (Metabion, Planegg/Steinkirchen, Germany) and 1x105 irradiated (100 Gy) lymphoma cells, which were derived from a λ-MYC tumor (8), twice in a two-weeks interval. After another 7 days, mice were sacrificed and spleens and lymph nodes were taken for further analyses (see below).

**2.2. Flow cytometry**

To obtain single cell suspensions, dissected spleens and lymph nodes were passed through a cell strainer (40 µm), followed by erythrocyte lysis, wash steps and filtration (35 µm). Surface staining was performed for 30 minutes at 4°C with monoclonal antibodies (mAbs) against CD4 (RM4-5; BD Pharmingen, Franklin Lakes, NJ, USA), CD8 (53-6.7 or H35-17.2; Thermo Fisher Scientific, Waltham, MA, USA), NK1.1 (PK136; BD Pharmingen) or PD-1 (J43; Thermo Fisher Scientific). Dead cells were excluded using the LIVE/DEADFixable Blue Dead Cell Stain Kit (Thermo Fisher Scientific). For intracellular staining, cells were fixed and permeabilized (Foxp3 Staining Buffer Set, Thermo Fisher Scientific), and proteins were labeled for 30 minutes at room temperature using mAbs against Foxp3 (FJK-16s), Ki67 (SolA15), Eomesodermin (Dan11mag), Blimp-1 (1B8), T-bet (eBio4B10), IFN-γ (XMG-1.2), TNF (MP6-XT22) or IL-10 (JES5-16E3; all from Thermo Fisher Scientific). To detect intracellular cytokines, cells were stimulated for 4 hours in complete cell culture medium with addition of 1 µg/ml PMA, 1 µg/ml ionomycin (both Sigma-Aldrich, Saint Louis, MO, USA) and 3 µg/ml Brefeldin A (Thermo Fisher Scientific) prior to staining. All flow cytometry data were acquired on an LSR II flow cytometer (BD) and analyzed using the Flowjo v10 software.

**2.3. *In-vitro* differentiation of Tr1 cells**

CD4+ T cells from spleens and lymph nodes of Great mice immunized as described above were enriched by using the EasySep mouse CD4+ T-Cell Isolation Kit (STEMCELL Technologies, STEMCELL Technologies, Vancouver, Canada) according to the manufacturer's protocol, followed by staining with the Fixable Blue Dead Cell Stain Kit (Thermo Fisher Scientific). Then, cells were suspended in PBS without Ca2+ and Mg2+ supplemented with 2 mM EDTA and 2% BSA in a concentration of 1.5x107 cells/ml. YFP+ cells were then sorted after dead-cell and doublet exclusion using a 100-µm nozzle and purity mode setting on a BD FACSAriaIIIu cell sorter. Re-analysis of YFP+ cells revealed a purity of >98 %.

For analyzing the differentiation of Tr1 cells *in vitro*, the highly enriched YFP+ cells (0,7x105/well) were incubated with unseparated wt or λ-MYC splenocytes (1x105/well) in complete cell culture medium (10% FCS) in the presence or absence of anti-IL-10 mAb (0.1 µg/ml) in round-bottom 96-well plates, which were coated with anti-CD3/anti-CD28 mAb (2 μg/ml; Core Facility mAb, Helmholtz-Zentrum München). To unambiguously identify after co-culture the T cells derived from immunized mice, the wt or λ-MYC splenocytes were additionally labeled with Cell Proliferation Dye (CPD) eFluor 450 (eBioscience) according to the manufacturer’s instructions.

Where indicated, the co-cultures were performed using CPD-labeled CD4+CD25- T effector cells that were isolated from spleens of immunized wt mice by using the CD4+ T-Cell Isolation Kit and the EasySep Mouse CD25 regulatory T Cell Positive Selection Kit (both from STEMCELL Technologies).

In all experiments, cells were harvested after 2 days and stained with LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Invitrogen) and mAbs against CD4+, Foxp3 and PD-1 or mAbs against IFN-γ and IL-10 after 4 hours of stimulation with PMA/Ionomycin and Brefeldin A. Cell samples were measured by flow cytometry.

**2.4. Statistics**

The unpaired or paired Student’s t test or Mann-Whitney test was used to assess differences between two independent groups. The results were expressed as means ± SEM. The survival benefit of λ-MYC mice that received anti-IL-10 therapy was analyzed using the log-rank test. Data were evaluated using the Prism 5.0 software (GraphPad). Significance was denoted as follows: \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001.

**3. Results**

**3.1. CD4+ T cells of the Th1 subtype accumulate in lymphoma but become increasingly exhausted**

-MYC mice constitutively express the *c-MYC* oncogene in a B cell-specific manner and develop B-cell neoplasias, which become manifest in spleens and lymph nodes about 60 to 130 days after birth (22). In the milieu of growing tumors, NK cells are initially activated but subsequently functionally impaired (8), which correlates with decreasing intratumoral IFN- concentrations (9). We therefore predicted that, at late tumor stages, secretion of Th1 cytokines by CD4+ T cells is also limited. Surprisingly, however, the percentage of IFN- and TNF-producing CD4+ cells was elevated in spleens of tumor-bearing mice (Fig. 1A, B) and even increased during disease progression (Fig. 1B). Possibly, these cells receive continued T cell receptor- (TCR-) dependent stimuli after initial Th1 differentiation.

Since chronic stimulation may result in T-cell exhaustion, we next examined the expression of PD-1 on tumor-infiltrating CD4+ T cells. Apart from PD-1negative cells, two other populations with low and high PD-1 levels, respectively, were distinguished (Fig. 2A). The latter two compartments were significantly expanded in spleens from tumor-bearing mice as compared to normal spleens (Fig. 2B). When IFN- expression was quantitated in these three subsets, the PD-1low fraction showed enhanced numbers of IFN--producing cells whereas the PD-1high subset displayed similar amounts as the PD-1negative population (Fig. 2C). This may indicate that chronic stimulation initially augments IFN- production and simultaneously triggers counterregulatory pathways, which is evidenced by continuous upregulation of PD-1 and – only in PD-1high cells – by a reversion of IFN- expression. IL-10 production was similarly induced in PD-1low cells, *i.e.* in those cells that had upregulated IFN- (Fig. 2D). In contrast to IFN- however, IL-10 further increased in correlation to rising PD-1 expression reaching its maximum level in PD-1high cells (Fig. 2C, D).

**3.2. Coexpression of IFN- and IL-10 in lymphoma-infiltrating CD4+ T cells**

Closer analyses of the cellular sources of IFN- and IL-10 in the tumor microenvironment revealed that, in the CD4+Foxp3- compartment, IL-10 was predominantly derived from IFN--expressing cells (Fig. 2E). The question arose whether the IFN-/IL-10 co-producers in -MYC spleens were *de novo* induced from Th0 cells or originated from Th1 cells that additionally upregulated IL-10 during tumor progression. Such cells were referred to as T regulatory type 1 (Tr1) cells (28-30).

Therefore, we first tested the IFN-+IL-10+ fraction for the transcription factor T-bet, a hallmark of Th1 differentiation (31, 32). Indeed, T-bet was expressed at high levels in this compartment (Fig. 3A). This subpopulation also showed increased expression of Blimp-1 and Eomesodermin in comparison to IFN-+IL-10- or to IFN--negative CD4+ T cells (Fig. 3B, C). Since both transcription factors are necessary for IL-10 induction in Th1 cells (33-35), the data indicate that Th1 cells may be converted to Tr1 cells in the lymphoma milieu.

To unequivocally show that IFN-/IL-10 co-expressing CD4+ T cells are derived from intratumoral Th1 cells, we selected a homogeneous IFN--producing CD4+ population and monitored the induction of IL-10 in these cells in an *in vitro* tumor milieu. To this end, T cells were derived from IFN- reporter with endogenous polyA tail (Great) mice, whose IFN--expressing cells can be readily detected and sorted by virtue of the yellow fluorescent protein (YFP), which is translated from a bicistronic IFN-/IRES/YFP mRNA (36). To induce lymphoma-reactive Th1 cells, Great mice were immunized with tumor cells from a MYC mouse along with CpG oligonucleotide (2, 5). Thereby, the frequency of IFN-/YFP+ CD4+ T cells in the periphery was raised about tenfold (not shown). It was established for MYC as well as for other tumors that CD4+ Th1 cells that are elicited by tumor cells in the presence of CpG oligonucleotide respond to these tumor cells specifically *in vitro* secreting high amounts of IFN- but no IL-4 (manuscript in preparation).

Following immunization of mice, YFP+ Th1 cells from spleen and lymph nodes were sorted and co-incubated with spleen cell suspensions from either normal wildtype (wt) or tumor-bearing MYC animals. IFN- expression was maintained even in the MYC tumor microenvironment (Fig. 4A). This shows that the lymphoma cells did not impair the *in vivo* generated T cells after Th1 polarization. Yet, IL-10 was induced in the Th1 cells during co-incubation. This effect was more pronounced in the *in vitro* MYC splenocyte milieu than in the presence of wt splenocytes (Fig. 4B). Most importantly, IL-10 upregulation was almost exclusively seen in the compartment of IFN-+ cells (Fig. 4A).

To analyze the factors driving conversion to the Tr1 type, the *in vitro* studies were repeated in the presence of an IL-10-neutralizing monoclonal antibody (mAb). In this setting, the IL-10 induction driven by the MYC splenocyte environment was reduced to the levels that were seen after incubation with wt splenocytes (Fig. 4B), indicating that IL-10 is required for Tr1 development.

**3.3. PD-1 upregulation is dependent on IL-10**

Since IL-10 expression correlated with PD-1 levels *in vivo* (Fig. 2D), we asked whether PD-1 was also upregulated during coculture with tumor suspensions. Upon incubation with MYC spleen cells, PD-1 expression by CD4+ Foxp3- T cells, which were derived from mice after -MYC-specific immunization, as outlined above, was indeed enhanced. This effect could be partly reversed by including an anti-IL-10 mAb (Fig. 4C). This shows that IL-10 contributes to PD-1 induction on Tr1 cells.

**3.4. Neutralization of IL-10 suppresses tumor growth *in vivo***

Taken together, the results suggested that IL-10 may inhibit tumor-suppressing Th1 responses in the -MYC model and that ablation of IL-10 may therefore have a beneficial effect for tumor control. To address this question, we treated -MYC mice *in vivo* with an IL-10-neutralizing mAb starting at time points before huge tumor burdens were apparent. This therapy significantly delayed the kinetics of tumor growth in comparison to the untreated controls (Fig. 5A). When T cells of treated animals were analyzed, PD-1 expression on CD4+Foxp3- cells was reduced (Fig. 5B) and the percentages of CD4+Foxp3- as well as CD8+ T lymphocytes were significantly increased (Fig. 5C, D). In the NK-cell compartment, cell counts, IFN- expression as well as proliferation were enhanced after anti-IL-10 therapy (Fig. 5E-G).

**4. Discussion**

The effects of IL-10 in malignant disease are a matter of controversy (14-21), and little is known on its relevance for immune regulation in B-cell lymphoma. We found that in sera of lymphoma-bearing -MYC mice, concentrations of IL-10 are strongly increased (37) and that malignant B cells are likely to be its primary source (24). This, in turn, seems to affect the IL-12/IL-10 ratio in DCs in favor of the latter (24). Although the progressive loss of IFN- secretion by intratumoral NK cells (8) as well as the imbalance of instructive cytokines in DCs anticipated a bias against Th1 differentiation, we observed an accumulation of IFN--producing Th1 cells in MYC lymphoma. This conundrum may be explained by initial Th1-directing signals provided by NK cells, which are strongly activated in early disease stages and only later lose IFN- expression (8). We recently identified several -MYC lymphoma-associated Ag peptides that may continuously stimulate the tumor-infiltrating CD4+ cells *via* TCR signals following Th1 polarization (25). Such peptides can be presented by tumor-infiltrating DCs, which also provide the requisite costimulatory signals (24).

The IFN- expression found in intratumoral CD4+Foxp3- T cells may be due to the chronic stimulation, which, however, may also give rise to their exhaustion as demonstrated by the induction of PD-1 and IL-10. Notably, the elevated IFN- levels found in the total CD4+Foxp3- population could be ascribed to cells upregulating PD-1 only moderately, while exclusively those T cells that displayed the highest PD-1 amounts showed reduced IFN- production. In contrast, expression of IL-10 was induced concomitantly to that of PD-1.

IL-10 was mainly produced in the IFN- fraction of the CD4+Foxp3- population. By exposing Th1 cells to a tumor microenvironment *in vitro*, we directly showed that this may reflect a counterregulatory mechanism, which results in the conversion of Th1 to Tr1 cells co-expressing IFN- and IL-10 (28-30) and upregulating PD-1 (30). Tr1 cells play a physiologic role for retracting ongoing proinflammatory responses (28). The *in vitro* result is in accordance with the phenotype of IFN-/IL-10-coproducing CD4+ T cells *in vivo*, where expression of the transcription factors Blimp-1 and Eomesodermin was found. Blimp-1 controls IL-10 expression in Th1 cells (35), induces expression of inhibitory receptors and is associated with T-cell exhaustion (review in 38). Eomesodermin activates IL-10 expression in cooperation with Blimp-1 and is necessary for Tr1 differentiation (34). Eomesodermin was suggested to unambiguously define Tr1 cells in humans (33).

Differentiation to the Tr1 phenotype in the -MYC model required pre-existing IL-10 (Fig. 4B), which was most likely derived from malignant B cells and DCs (24). On the other hand, IL-10 derived from CD4+ T cells having undergone Tr1 conversion is also able to dampen potentially lymphoma-suppressing Th1 responses (*e.g.* 39). In this context, IL-10 may be effective in a paracrine as well as autocrine manner.

As IL-10 obviously contributes to tumor immune escape in the lymphoma model, it can be used as a target for immunotherapy. Ablation of IL-10 indeed protected from the exhaustion of CD4+ effector cells, ameliorated the numbers of CD4+ and CD8+ T cells (Fig. 5B-D) and, most importantly, led to a significant survival benefit of -MYC animals (Fig. 5A). Thus, delivery of IL-10-neutralizing mAbs might be an additional option for immunotherapy of lymphoma in the clinic, possibly in combination with other regimens. In the situation of HIV infection, blocking of IL-10 together with immune checkpoint inhibition also restored CD4+ T-cell functions and thereby even stimulated effector systems of the innate immune system (40). In lymphoma-bearing animals, anti-IL-10 mAb treatment similarly enhanced the numbers and the function of NK cells (Fig. 5E-G). Deciphering the underlying mechanisms has to await future studies. Most likely, the therapeutic success requires several components of the immune system, which are modulated by anti-IL-10 mAb treatment and which have to cooperate to provide the survival benefit observed.

Apart from B cells, DCs and Tr1 cells, Foxp3+ Treg cells are another source of IL-10 in -MYC lymphoma (25). As recently shown, depletion of this T-cell subset also induces effective tumor control (25). Although the mechanisms mediating Treg cell-dependent immunosuppression are not yet fully elucidated in this model, it is most likely that Treg cells also contribute to exhaustion of T effector cells *via* IL-10 secretion (14). However, the immunosuppressive effects of Foxp3+ Treg cells might also be related to additional mechanisms such as cell contact-dependent inhibition of effector T cells or trapping of IL-2 (manuscript in preparation). In the present work, we focus on CD4+Foxp3- Th1 and Tr1 cells in lymphoma. Further studies are required to unravel the specific significance of Foxp3+ Treg cells and other Foxp3- cellular subsets for immune regulation in this disease and to eventually develop novel approaches of cancer therapy based on inhibiting immunosuppressive pathways.

**Authors´ contributions**

Y.M., V.B., T.R., F.A., N.H. and T.P.H. performed experiments and analyzed data; Y.M., V.B. and N.H. designed the figures; M.R. and R.M. conceived and supervised the study and wrote the manuscript.

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**Conflict-of-interest disclosure**

The authors have no conflicts of interest to disclose.

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**Figure legends**

**Figure 1.** Production of Th1 cytokines by tumor-infiltrating CD4+ Foxp3- T cells in spleens of -MYC mice. **A,** Representative dot plots showing the expression of IFN- and TNF by CD4+ T cells from mice bearing heavy tumor burdens in comparison to wt animals. **B,** Percentages of IFN-- and TNF-producing cells in the CD4+ population in correlation with disease progression. Mice were ascribed to the early disease stage when there was incipient tumor growth (deteriorating general state without visible tumor burdens), while in the late stage, mice showed heavy tumor masses. Compilation of 6 to 13 mice per group.

**Figure 2.** Exhaustion of CD4+ T cells in -MYC tumors. **A,** Distribution of PD-1negative, PD-1low and PD-1high cells in the CD4+Foxp3- T-cell population of tumor-infiltrated -MYC spleens. **B,** Increased PD-1high and PD-1low CD4+ cell fractions in spleens of tumor-bearing animals compared to spleens from wt mice. 7 to 9 mice were included in each group. **C,** Percentages of IFN--producing T cells in CD4+ populations expressing different levels of PD-1 in spleens from tumor-bearing -MYC animals. **D,** Correlation of IL-10 expression with PD-1 surface levels in CD4+ T cells from -MYC spleens. 3 to 5 mice were included in each column. **E,** Co-expression of IFN- and IL-10 in CD4+ T cells from tumor spleens. Only in the IFN--negative, but not in the IFN-+ fraction, IL-10 was derived from Foxp3+ Treg cells in significant amounts (41). In the results shown here, Foxp3+ cells were excluded.

**Figure 3.** Expression levels of selected transcription factors in the IFN--IL-10-, IFN-+IL-10- and IFN-+IL-10+ fraction of tumor-infiltrating CD4+Foxp3- T cells. **A,** Expression of T-bet, which is a hallmark of Th1 differentiation. **B, C,** Expression levels of Blimp-1 and Eomesodermin, which are necessary for IL-10 induction in Th1 cells. Means of up to 13 mice are shown in each column. MFI: mean fluorescence intensity.

**Figure 4.** Conversion of Th1 cells to Tr1 cells in a lymphoma milieu *in vitro*. Purified IFN--expressing Th1 cells induced by *in vivo* immunization with -MYC tumor cells were incubated with suspensions of normal or tumor-infiltrated spleens. For details see text. **A,** Exemplary dot plot showing the expression of IFN- and IL-10 in Th1 cells after incubation with -MYC splenocytes. **B,** Comparison of wt and lymphoma spleens in terms of inducing IL-10 expression in purified IFN--producing Th1 cells. In the presence of an anti-IL-10 mAb, IL-10 induction in IFN--producing Th1 cells in the -MYC tumor microenvironment is dampened. Compilation of three experiments. **C,** PD-1 upregulation in CD4+ T cells, which were derived from mice after -MYC-specific immunization, in an *in vitro* lymphoma microenvironment compared to a normal wt spleen milieu. The effect was partly reversed by mAb-mediated IL-10 neutralization (n=7).

**Figure 5.** Effects of treating -MYC mice with anti-IL-10 mAb. **A,** Survival times of -MYC mice that did or did not receive anti-IL-10 mAbs. **B,** Reduced PD-1 expression on CD4+Foxp3- cells from spleens of -MYC animals having undergone anti-IL-10 therapy. **C, D,** Percentages of CD8+ and CD4+Foxp3- T cells in spleens of treated *versus* control mice. **E,** Fractions of NK cells in the lymphocyte population of treated and untreated mice. **F,** IFN- production by NK cells after anti-IL-10 therapy. **G,** Proliferation of NK cells after anti-IL-10 treatment. All parameters shown were determined in spleens of mice that had to be euthanized due to tumor growth. 5 to 12 animals were analyzed in each group.