Fms-Like Tyrosine Kinase 3–Based Immunoprophylaxis against Infection Is Improved by Adjuvant Treatment with Anti–Interleukin-10 Antibody

Lopamudra Das,1,2 Jennifer DeVecchio,3 and Frederick P. Heinzel2,3
1Center for Global Health and Diseases and 2Department of Medicine, Case Western Reserve University, and 3Medical Research Service, Louis Stokes VA Medical Center, Cleveland, Ohio

Background. Fms-like tyrosine kinase 3 ligand (Flt3L) expands dendritic-cell populations in vivo and protects against microbial infection in healthy and immunocompromised hosts. Approaches for optimizing the protective effects of Flt3L in vivo are not well known.

Methods. BALB/c mice were treated for 9 days with 10 μg of recombinant (r) Flt3L with or without the addition of 250 μg of anti–interleukin (IL)–10 antibody on day 9. After Leishmania major infection, disease progression was determined by measuring cutaneous lesions. Production of IL-12 and interferon (IFN)–γ were determined.

Results. Flt3L pretreatment increased the synthesis of CD40-inducible IL-12 p40 but not of bioactive p70. Coculture with anti–IL-10 antibody increased p70 production. Combined Flt3L and single-dose anti–IL-10 antibody pretreatment improved lesion cure rates from 40% to 87% relative to mice pretreated with rFlt3L only ($P<.01$, $\chi^2$ test) and increased T helper 1 (Th1)–type cytokine production 4 weeks after infection but did not cure Rag-2– and IFN-γ–knockout BALB/c mice. Flt3L and anti–IL-10 antibody pretreatments increased frequencies of IL-12– and IFN-γ–secreting cells 2 and 4 days after infection. Both natural killer and CD4+ cells contributed to increased early IFN-γ production.

Conclusion. A single dose of anti–IL-10 antibody significantly improves Flt3L immunoprophylaxis against infection mediated by Th1-type adaptive responses.

The Fms-like tyrosine kinase 3 (Flt3) receptor is expressed on myeloid and lymphoid progenitor cells, and it controls the growth of dendritic cells (DCs) and other cells of the innate immune system [1]. Treatment with soluble Flt3 ligand (Flt3L) dramatically increases numbers of DCs in vivo [2]. Flt3L–treated mice and primates demonstrate improved immunologic functions related to accessory cell functions, including both antigen presentation and the production of immunoregulatory cytokines [3–5]. As a result, Flt3L pretreatment increases vaccine responses [6] and enhances resistance to acute bacterial and viral infections in both healthy and immunocompromised hosts [7–9]. These results suggest that a combination of innate and adaptive immune mechanisms related to DCs and NK cells contribute to these outcomes.

We previously reported that pretreatment with recombinant (r) Flt3L for 10 days improved resistance to progressive cutaneous infection with the intracellular protozoan parasite Leishmania major [10]. In that high-inoculum model of progressive cutaneous leishmaniasis, disease-susceptible BALB/c mice did not control infection because of biased expansions of Th2-type CD4 cells and a disruption of leishmanicidal responses normally mediated by Th1 cell–derived interferon (IFN)–γ [11]. Although it is not representative of normal cutaneous leishmaniasis in humans, that model is well-characterized and stringent for testing the immunologic effectiveness of Th2-deviating therapies in chronic infection. For instance, treatment with r interleukin (IL)–12 or anti–IL-4 antibody during the first week of in-
fection promoted unipolar Th1 responses and cure of disease in >90% of mice [12, 13]. In contrast, only 40% of rFlt3L-pretreated BALB/c mice eventually controlled lesion size. The development of Th1-biased immunity was incomplete or delayed, although cured mice were resistant to reinfection. These observations and the increasing experimental use of rFlt3L as anti-infective immunoprophylaxis led us to study practical interventions that would improve the antimicrobial and Th1-promoting effects of rFlt3L. We show that a single injection of anti–IL-10 antibody markedly improves cure rates in rFlt3L-pretreated mice and that this is associated with increased early IL-12 and IFN-γ responses that include both innate and adaptive contributions.

MATERIALS AND METHODS

Cytokines, antibodies, and other reagents. Human (h) rFlt3L produced by CHO cells was generously provided by Amgen. Anti–IL-10 antibody (JES 2A5), anti–major histocompatibility complex (MHC) II (TIB120), and anti-CD40 (FGK 45) monoclonal antibodies (MAbs) were purchased from BioExpress. Antibodies, rFlt3 ligand, PBS–0.1% mouse serum albumin (MSA; Sigma), and cell culture medium used in these studies were shown to be endotoxin free according to the results of a Limulus lysate assay (E-Toxate; Sigma). Salmonella enteriditis lipopolysaccharide (LPS; Sigma) and mouse rIFN-γ (R&D) were used for stimulation in splenocyte cultures.

Mice. Four- to 6-week-old female wild-type (wt) Rag-2−/− and IFN-γ−/− BALB/cBYJ mice were purchased from Jackson Laboratories and housed at the Case Western Reserve University animal facility under specific pathogen–free conditions. Mice laboratories and housed at the Case Western Reserve University

Parasites, antigens, and mouse infection. L. major (World Health Organization strain WHOM/3-173) was grown in M199 medium (Gibco Laboratories) that contained antibiotics, supplemental glutamine, and 30% fetal calf serum/HEPES (pH 7.4; Hyclone Laboratories), as described elsewhere [10]. Soluble leishmanial antigen (SLA) was prepared from metacyclic promastigotes as described elsewhere [13]. Stationary-phase (metacyclic) promastigotes were injected into both hind feet of recipient mice at a dose of 2 × 10⁶ organisms/footpad, and the course of infection was monitored by weekly measurements of footpad thickness. Cure was defined as reversal of footpad swelling to ≤3 mm 4 weeks after infection—a time at which either progression or resolution of disease has become irreversible, according to the results of previous studies that lasted up to 14 weeks.

Culture of spleen and lymph-node cells. Spleens and lymph nodes were harvested from uninfected or infected mice when indicated. Spleens were incubated, at room temperature for 20 min, in Hanks' balanced salt solution (HBSS)/Ca²⁺/Mg²⁺ that contained 200 U/mL collagenase IV (Boehringer-Mannheim). The suspensions were centrifuged at 200 g for 15 min, and red cells were lysed by use of hypotonic ACK lysis buffer (150 mmol/L ammonium chloride, 10 mmol/L potassium carbonate, and 0.1 mmol/L EDTA [adjusted to pH 7.4]) as needed for spleen cells. Cell suspensions were then washed 3 times in HBSS without Ca²⁺/Mg²⁺, counted, and suspended in Dulbecco’s modified Eagle medium (DMEM)/10% fetal bovine serum (FBS; DMEM supplemented with 100 μg/mL penicillin and streptomycin, 2 mmol/L glutamine, 0.1 mmol/L nonessential amino acids, 10% FBS [buffered at pH 7.4], and 10 mmol/L HEPES). Suspended cells were aliquoted into flat-bottomed 96-well culture plates at 10⁴ cells/well and cultured for 48 h. Cells were cultured in DMEM/10% FBS culture medium only or in medium that contained SLA at 10 μg/mL.

Cytokine ELISA and enzyme-linked immunospot (ELISPOT) assays. Culture supernatants were assayed for concentrations of murine IL-12 p40, IL-4, and IFN-γ by use of antibody reagents from BD Pharmingen and ELISA techniques described elsewhere [10]. IL-12p70 was assessed by use of an ELISA kit from R&D Systems. ELISPOT plates (Immunospot M200; Cellular Technology) were coated overnight at 4°C with capture antibodies for IL-12 p40 or IFN-γ (BD-Pharmingen), then washed and blocked with PBS/1% bovine serum albumin for 1 h at room temperature. After washing, freshly isolated lymph-node cells were plated at 10⁵ cells/well in 3 replicate wells in serum-free HL-1 medium (BioWhittaker) supplemented with L-glutamine and penicillin/streptomycin. After a 24-h culture at 37°C, cells were removed by 3 washes with PBS and 4 more washes with PBS/0.05% Tween-20 (PBST). Cytokine-specific biotinylated detection antibodies were added for an overnight incubation at 4°C, and plates were washed 3 times with PBST followed by a 2-h incubation at room temperature with streptavidin-alkaline phosphatase conjugate (DAKO) at 1:1000 dilution. Color was developed by use of nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Sigma); then plates were washed with distilled water and air dried. Spots were quantified by use of the Series-1 Immunospot image analyzer (Cellular Technology).

Depletion of specific lymph-node cell populations. Lymph-node cells from treated and untreated mice were depleted of CD4 cells and/or NK cells by treating the cells for 20 min at 4°C with magnetic beads specific for surface CD4 or DX5 markers (Miltenyi Biotec), followed by passage over a magnetized column. Depleted and control populations of cells were cultured at 10⁶ cells/well in ELISPOT assay plates.

Statistics. Quantitative differences between multiple de-
results

increase in splenocyte production of IL-12 p40, but not of IL-12 p70, by Flt3L in response to CD40 stimulation. As has been shown elsewhere [10], splenocytes from rFlt3L-treated BALB/c mice produced up to 7-fold more IL-12 p40, constitutively or in response to an activating anti-CD40 antibody than did splenocytes from saline-pretreated mice (figure 1). In contrast, anti-CD40–induced production of IL-12 p70 increased only 2-fold relative to either anti-CD40–stimulated control splenocytes or unstimulated Flt3L-pretreated cells. This was stimulus dependent—Flt3L-pretreated splenocytes cultured with LPS and rIFN-γ produced nearly 10-fold more IL-12 p70 than did saline-pretreated splenocytes. These changes were similar when p70 responses were evaluated either in absolute concentrations or as a percentage of total IL-12. Underproduction of IL-12 p70 relative to p40 was also observed in cultures of popliteal and inguinal lymph-node cells from rFlt3L-pretreated mice (data not shown).

increase in CD40-induced IL-12 p70 production in cultures of rFlt3L-pretreated spleen by anti–IL-10 antibody. Compared with anti-CD40 antibody alone, the addition of neutralizing anti–IL-10 antibody increased IL-12 p70 production in cultured rFlt3L-pretreated splenocytes by 10- and 6-fold for absolute and relative concentrations, respectively (figure 1). Anti–transforming growth factor (TGF)–β antibody did not augment anti-CD40–simulated IL-12 production. By way of comparison, anti–IL-10 antibody was comparable to rIFN-γ in enhancing CD40-inducible IL-12 p70 production by rFlt3L-pretreated splenocytes.

increase in the disease-protective effects of rFlt3L in L. major–infected BALB/c mice after cotreatment with anti–IL-10 antibody. Groups of saline- and rFlt3L-pretreated BALB/c
mice were injected ip with 250 μg of neutralizing anti–IL-10 IgG or rat IgG the day before subcutaneous injection with L. major in both hind feet (figure 2A). After week 4, both rFlt3L and rFlt3L/anti–IL-10 antibody–pretreated mice showed significant reductions in footpad size, compared with saline- and anti–IL-10 antibody–pretreated control mice, and rFlt3L/anti–IL-10 antibody–pretreated mice showed significant reductions relative to rFlt3L-pretreated mice starting at week 6 (P < .05, ANOVA). In a series of 5 experiments, 13 (42%) of 31 disease-susceptible BALB/c mice treated with rFlt3L alone resolved cutaneous lesions by week 8 of infection, compared with 0 of 32 control mice (P < .01, χ² test). The inclusion of a single dose of anti–IL-10 antibody at the end of Flt3L treatment significantly increased cure rates to 88% (49/56 mice; P < .01 vs. rFlt3L treatment only). Treatment with anti–IL-10 antibody alone delayed the progression of disease, compared with that in saline-pretreated control mice, but lesion sizes progressed in all mice and were no different than control footpad sizes by the fifth week of infection (P = .54, ANOVA).

T and B cell–dependent cure of leishmaniasis in rFlt3L- and anti–IL-10 antibody–pretreated mice. Because Flt3L expands innate immune effector cells, such as DCs and NK cells, we tested whether rFlt3L/anti–IL-10 antibody–expanded innate immunity might be sufficient to restrict lesion development in T and B cell–deficient Rag-2−/− BALB/c mice. Again, Flt3L/anti–IL-10 antibody–pretreated wt mice controlled lesion development to <3 mm throughout the 7-week study, whereas the lesions of mice treated with anti–IL-10 antibody progressed to >5 mm and developed ulcerations. However, all Rag-2−/− mice developed progressively enlarging lesions regardless of whether pretreatment was with rFlt3L/anti–IL-10 antibody or anti–IL-10 antibody alone, but lesion development was slower than that observed in control wt mice (figure 2B). These findings show that rFlt3L-expanded innate cellular immunity alone is insufficient to mediate the cure of leishmaniasis.

Increased Th1 bias during infection after combined pretreatment with rFlt3L and anti–IL-10 antibody. We previously reported that the partial protection of rFlt3L alone was associated with incomplete or delayed conversion from IL-4– to IFN-γ–dominant lymph-node cytokine responses, compared with naturally resistant C57BL/6 mice. In the present studies, both rFlt3L- and rFlt3L/anti–IL-10 antibody–treated BALB/c mice significantly decreased lymph-node IL-4 synthesis by 4 weeks after infection relative to that in control mice (P < .01, figure 3A). However, IFN-γ production was increased significantly only in mice pretreated with both rFlt3L and anti–IL-10 antibody. Neutralizing anti–MHC IId MAb (M5/114; 10 μg/mL) added to replicate cultures blocked ~85% of IFN-γ and
90% of IL-4 steady-state production. This is consistent with the results of previous studies, which showed that CD4+ cells were major sources of these cytokines during murine leishmaniasis. Cure of infection in rFlt3L/anti–IL-10 antibody–treated mice was IFN-γ dependent, given that only wt BALB/c mice, and not IFN-γ−/− BALB/c mice, were able to control lesion development (figure 3B).

Effects of rFlt3L and/or anti–IL-10 antibody pretreatment on early IL-12 and IFN-γ responses after infection. Within days of L. major infection in mice, frequencies of both IL-12 p40– and IFN-γ–producing cells increase in the draining lymph nodes [14]. Disease-resistant mice develop higher levels of production than do susceptible BALB/c mice during the first week, and these early responses may affect subsequent T cell responses and disease outcomes. Lymph-node IL-12 p40 and IFN-γ frequencies according to ELISPOT assay were compared across 4 groups of infected BALB/c mice that had been pretreated singly with saline, anti–IL-10 antibody or rFlt3L, or combined rFlt3L and anti–IL-10 antibody (figure 4). We confirmed that IL-12 p40 spot-forming responses increased 2 and 4 days after infection in saline-pretreated BALB/c mice, at levels ∼10-fold greater than those observed in uninfected lymph nodes. Anti–IL-10 antibody pretreatment alone had no significant effect on IL-12 p40 production. However, rFlt3L- or rFlt3L/anti–IL-10 antibody–pretreated mice produced 5-fold higher IL-12 p40 frequencies than did their respective saline- or anti–IL-10 antibody–pretreated controls (P < .05; ANOVA). Although SLA suppressed p40 frequencies at 2 days of infection, this was not observed at 4 days. In 3 other experiments, both medium- and SLA-cultured cells were similarly increased in Flt3L-pretreated mice.

Starting 2 days after infection, IFN-γ ELISPOT frequencies in all experimental groups were increased 3–4-fold, relative to those in uninfected mice (figure 4). Otherwise, there were no significant differences between groups or culture conditions.
By 4 days after infection, frequencies of IFN-γ-producing cells were significantly increased in antigen-stimulated cultures, compared with medium control cultures. Furthermore, IFN-γ frequencies were significantly greater in Flt3L- and rFlt3L- and anti–IL-10 antibody–pretreated mice than in their respective saline and anti–IL-10 antibody–pretreated controls. Although pretreatment with anti–IL-10 antibody alone did not affect IL-12 p40 frequencies relative to controls, anti–IL-10 antibody did significantly increase frequencies of IFN-γ–producing cells at day 4 relative to those in saline-pretreated controls. These findings suggest that pretreatment with rFlt3L is sufficient to increase an early and spontaneous IL-12 p40 response during infection, which is associated with increased antigen-specific IFN-γ responses. Pretreatment with anti–IL-10 antibody may independently enhance antigen-inducible IFN-γ responses.

Cellular sources of early cytokine production in pretreated mice. We next determined the relative role of innate and adaptive immunity in the early cytokine response of rFlt3L-pretreated mice. wt and Rag-2-/- BALB/c mice pretreated with rFlt3L and anti–IL-10 antibody spontaneously produced ~10-

Figure 4. Distinct effects of recombinant Fms-like tyrosine kinase 3 ligand (rFlt3L) and anti–interleukin (IL)–10 antibody pretreatment on lymph-node frequencies of IL-12 and interferon (IFN)–γ–producing cells during the first week of infection. Four groups of 5 BALB/c mice each were treated with saline, anti–IL-10 antibody, rFlt3L, or both, as indicated on the Y-axis. For each group, draining popliteal lymph node cells from days 2 and 4 after infection were cultured for 48 h with medium or soluble leishmanial antigen (SLA; 10 μg/mL) in enzyme-linked immunospot (ELISPOT) assay plates. The frequencies of IL-12 p40–producing (left) and IFN-γ–producing (right) cells are shown as the mean ± SE no. of spot-forming cells per 10^6 cells of triplicate cultures of pooled lymph-node cells. Included are comparisons of frequencies of spot-forming cells at days 2 (top) and 4 (bottom) after infection. Included in the day 2 analysis are results from uninfected lymph-node cultures, which produced <10 spots of either cytokine/10^6 cells. IL-12 p40 and IFN-γ frequencies significantly increased in rFlt3L- and rFlt3L- and anti–IL-10 antibody–pretreated mice vs. saline and anti–IL-10 antibody–pretreated controls, as indicated by brackets and asterisk (P<.05, analysis of variance [ANOVA]). Within-group increases in IFN-γ frequency for SLA- vs. medium-stimulated cultures are indicated by 2 asterisks (P<.05, ANOVA).
Figure 5. Promotion by Fms-like tyrosine kinase 3 ligand (Flt3L) of early interferon (IFN)-γ production in T and B cell–deficient BALB/c mice after Leishmania major infection: the role of NK and CD4+ cells. A, Groups of 5 wild-type or RAG-2 knockout (RAG KO) BALB/c mice were treated with saline (Control) or with recombinant (r) Flt3L and anti–interleukin (IL)–10 antibody, as described in Materials and Methods. The frequencies of IL-12 p40– and IFN-γ–producing cells per 10^6 lymph-node cells were determined at days 2 and 4 after infection in medium alone (white squares) or medium that contained soluble leishmanial antigen (SLA; 10 μg/mL) (black squares). Shown are mean ± SE of triplicate cultures of pooled lymph-node cells. Brackets with a single asterisk indicate significant increases in IL-12 p40 and IFN-γ frequencies for rFlt3L- and anti–αIL-10 antibody–pretreated mice vs. their respective saline-pretreated controls ( , analysis of variance). Two asterisks indicate significantly increased IFN-γ frequencies in SLA- vs. medium-stimulated cultures. B, BALB/c mice treated as described in Materials and Methods and lymph-node cells assayed by enzyme-linked immunospot assay before or after depletion of CD4+ (CD4+) or DX5+ NK (NK+) lymphocytes or both populations (CD4+ NK+) by use of specific magnetic microbeads. Cells were cultured in equal nos. (5 × 10^6/μL) with medium or SLA. Shown are the mean ± SE of values from triplicate cultures of pooled lymph-node cells. An asterisk indicates a significant reduction in IFN-γ frequencies in CD4/NK-depleted cultures vs. the respective predepletion controls.
and 20-fold more IL-12 p40-producing lymph-node cells (535.3 ± 9.7 vs. 50 ± 3.6; P < .01) than did their respective untreated controls 2 days after infection (P < .05) (figure 5A). In 3 of 5 experiments in which rFlt3L- and anti–IL-10 antibody–pretreated mice were used, increased IL-12 frequencies on day 2 preceded an increase in antigen-specific IFN-γ responses at day 4 in rFlt3L- and anti–IL-10 antibody–pretreated BALB/c mice, relative to those in saline-pretreated controls. As expected, antigen-specific IFN-γ production was not seen in Rag-2−/− mice. Instead, rFlt3L- and anti–IL-10 antibody–pretreated Rag-2−/− mice generated increased frequencies of cells that spontaneously produced IFN-γ relative to saline-pretreated Rag-2−/− controls (P < .05) (108 ± 17.6 vs. 11.3 ± 2.4, respectively; P < .01).

Because these findings suggested that rFlt3L expanded both T and NK cell sources of early IFN-γ, we determined the extent to which rFlt3L- and anti–IL-10 antibody–enhanced IFN-γ production was dependent on the presence of CD4+ cell and/or DX5+ NK cells in the draining lymph-node culture. Magnetic beads were used to deplete at least 75% of CD4+ and DX5+ NK cells, relative to the starting lymph-node population, before ELISPOT assay (figure 5B). Isolated depletions of CD4+ or NK cells did not change IFN-γ responses by >25% either 2 or 4 days after infection. However, the combined depletion of CD4+ and NK cells markedly impaired SLA-induced IFN-γ synthesis at day 2 for rFlt3L- and anti–IL-10 antibody–pretreated mice and for both control and rFlt3L- and anti–IL-10 antibody–pretreated mice at day 4 (P < .05). These findings suggest that both NK and CD4+ cells contribute independently to IFN-γ production early during murine leishmaniasis and that these contributions are both increased after pretreatment with rFlt3L and anti–IL-10 antibody.

DISCUSSION

rFlt3L has emerged as a DC-targeted immunotherapy that augments anti-infective responses in healthy hosts and reduces infectious susceptibility in immunodeficient hosts. Flt3L also assists in the reconstitution of innate immunity in mice after bone-marrow transplantation and may improve tumor immunity, which suggests a wider range of potential clinical uses [15–17]. Our studies contribute to this emerging form of therapy by showing a practical intervention for increasing both the early and delayed effects of rFlt3L on Th1 cell development in chronic infection. Specifically, we observed that the addition of anti–IL-10 antibody increased the production of IL-12 p70 by anti-CD40–stimulated spleen cells from rFlt3L-pretreated mice. We used CD40 activation as a readout, because CD40/CD40L interactions mediate IL-12 production during live infection [18, 19]. The activating anti-CD40 antibody used has a bioactivity that is comparable to soluble forms of native CD40L. Although both IL-10 and TGF-β can specifically suppress IL-12 p70 production [20, 21], only anti–IL-10 antibody was active in these studies. IL-10 was present in low levels in both healthy and rFlt3L-pretreated spleen culture without a significant difference, which suggests that inhibition was constitutive and sufficient to significantly impair Flt3L-expanded IL-12 synthesis.

A single injection of anti–IL-10 antibody doubled protection in rFlt3L-pretreated mice but did not protect saline-pretreated BALB/c mice. Previously, the curing of BALB/c leishmaniasis required either the use of IL-10−/− mice or prolonged therapy with anti–IL-10 or anti–IL-10R antibodies [22, 23]. Our successful use of single-dose anti–IL-10 antibody suggests that the beneficial effects were likely mediated early and uniquely on rFlt3L-expanded cell populations, which persist only up to 1 week after treatment. Although we have not identified a source of IL-10 in these studies, an early constitutive source of IL-10 in murine leishmaniasis includes CD4+CD25+ regulatory cells [24]. IL-10 can also be induced in macrophages when their Fc receptors are engaged by antibody-coated parasite later in infection [22].

We confirmed that rFlt3L therapy gave rise to MHC II–dependent T cell responses after 4 weeks of infection that produced approximately one-half the IL-4 produced by control infected mice. When anti–IL-10 antibody was also administered, IFN-γ synthesis was also increased. Deviation from Th2 to Th1 cytokine polarity is essential for recovery from L. major infection, because IL-4 and IL-13 antagonize IFN-γ–dependent and inducible nitric oxide synthase–based killing of these intracellular parasites [25]. Consistent with a classic Th1-based mechanism of cure, pretreatment with rFlt3L and anti–IL-10 antibody did not protect mice that were either IFN-γ or T cell deficient. Although an expanded CD4+ Th1 population is consistent with disinhibited IL-12 p70 bioactivity after the neutralization of IL-10, the ELISPOT technique could not be adapted to measure physiologic IL-12 p70 responses early during infection, and we do not confirm that IL-12 p70 production increases in the draining lymph-node population during infection.

We observed increased innate immune production of IFN-γ during the first week of infection in rFlt3L-pretreated mice, as shown by increased IFN-γ synthesis in Rag2−/− mice. Although an intact adaptive immune system was required for cure in rFlt3L- and anti–IL-10 antibody–pretreated mice, early innate cytokine production regulates Th1 development in other models of infection or adjuvant-guided immunization [26]. We confirm that NK cells produce IFN-γ early during murine leishmaniasis, but the physiologic significance of this response is unclear. For instance, NK cell–derived IFN-γ is not necessary for Th1 expansion and cure of infection in resistant C57BL/6 mice [27, 28]. In contrast, early IFN-γ production affects the development of Th1 responses in highly resistant C3H mice, which maintain larger NK cell populations than do C57BL/6 or BALB/c mice [29]. In this respect, rFlt3L may be modulating the BALB/c innate immune response to infection to more closely resemble
that of highly resistant C3H mice. However, neither CD4+ nor NK cells alone were sufficient to generate an increased IFN-γ response in rFlt3L- and anti–IL-10 antibody–pretreated mice. An independent protective role of expanded NK cell function cannot be concluded from the results of this study.

We conclude that the anti-infective potential of rFlt3L can be optimized by transient neutralization of IL-10. Although these observations are limited to a model of disease in which IL-12 p70 is CD40 dependent, this approach may be applicable to infections associated with strong innate immune activation and in which IL-10 has known suppressive effects. With this modification, rFlt3L may also better recruit strong anti-microbial responses in hosts that are compromised by inherit or acquired defects in numbers of DCs or DC-dependent IL-12 synthesis, such as which occurs after trauma or in HIV infection [8, 30, 31]. However, rFlt3L is likely to be used in settings where enhanced adaptive immunity is desired, in which case suppression by IL-10 becomes a significant variable affecting outcome. Further study of anti–IL-10 antibody adjuvant therapy for Flt3L-enhanced vaccination, antimicrobial prophylaxis, bone-marrow immune reconstitution, and cancer immunotherapy is indicated.

Acknowledgments

We gratefully acknowledge the support of Amgen, for providing the reagent recombinant human Fms-like tyrosine kinase 3 ligand (from CHO cells); and Meetha Pai Gould, Jennifer Greene, Jeffrey Auletta, Gopal Yadavalli, and Kara Martin, for their valuable help and discussions.

References

16. Pawlowska AB, Hashino S, McKenna H, Weigel BI, Taylor PA, Blazar BR. In vitro tumor-pulsed or in vivo Flt3 ligand-generated dendritic cells provide protection against acute myelogenous leukemia in non-transplanted or syngeneic bone marrow-transplanted mice. Blood 2001; 97:1474–82.
