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Inflammation Research

Effect of slow release IL-12 and IL-10 on inflammation, local macrophage function and the regional lymphoid response during mycobacterial (Th1) and schistosomal (Th2) antigen-elicited pulmonary granuloma formation

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Abstract. *Objective and Design:* This study examines the local and regional effects of exogenously administered interleukins 10 (IL-10) and 12 (IL-12) on pulmonary granulomas mediated by Th1/type 1-(IFN-γ) and Th2/type 2-(IL-4, IL-5) cytokines.
Materials and Treatments: Granulomas (GR) were induced in presensitized CBA mice by embolization of beads coated with *Mycobacteria tuberculosis* or *Schistosoma mansoni* egg antigens. Before challenge, osmotic pumps distributing IL-10 or IL-12 (50 μg/kg/day) were implanted intraperitoneally, then GR and draining lymph nodes were examined 4 days.
Methods: GR sizes and composition were determined by morphometry and differential analysis. Isolated GR macrophages and draining lymph nodes were assessed for cytokine production by ELISA.
Results: IL-10 did not effect GR sizes but reduced neutrophils in type 1 GR. IL-12 minimally reduced type 1 GR but decreased the type 2 lesion by up to 70%, primarily curtailing eosinophils. Type 2 GR macrophages were unaffected but type 1 were impaired by IL-10. Conversely, type 1 GR macrophages were more resistant to IL-12 while type 2 showed enhanced IL-10, IL-12 and TNF, but reduced MCP-1 production. In lymph nodes, IL-10 caused paradoxical effects, enhancing IFN-γ in the type 1 and decreasing Th2 cytokines in the type 2 response. Exogenous IL-12 profoundly augmented IFN-γ and abrogated type 2 cytokines while inhibiting intrinsic IL-12 production in lymph nodes.
Conclusion: These findings provide novel information regarding cytokine regulation and the effects of systemic cytokine therapy.

Key words: Interleukin 10 – Interleukin 12 – Granuloma – Immunotherapy

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Introduction

Interleukins 10 (IL-10) and 12 (IL-12) are thought to be critical cytokines regulating the nature and extent of T helper (Th) cell functions and inflammatory events [1, 2]. IL-10, produced by macrophages and Th2 cells, is known to inhibit the production of a number of proinflammatory cytokines such as interferon-γ (IFN-γ) interleukin-1 (IL-1) and tumor necrosis factor (TNF) [3–5]. IL-12, produced by macrophages and lymphocytes, promotes IFN-γ production and is known to augment natural killer and Th1 cell-mediated immune reactions [6, 7]. It is not surprising that these cytokines are considered potential therapeutic agents for manipulation of immune and inflammatory responses. For example, recombinant IL-10 combined with IL-4 can inhibit cell-mediated inflammation [8]. In contrast, recombinant IL-12 enhanced Th1 cell-mediated resistance to intracellular parasites such as *Leishmania* [9, 10] and inhibits Th2 cell-mediated responses to schistosome eggs [11, 12].

To date, studies using cytokines as immunopharmacologic agents generally use a variety of nonstandardized disease models with variable object parameters. Understanding the potentially complex in vivo effects of these treatments would be enhanced by using standardized approaches. We recently described standardized murine models of type 1 (Th1) and type 2 (Th2)-cytokine-mediated pulmonary granulomatous (GR) inflammation elicited with polymer beads coated with purified protein derivative of *Mycobacteria tuberculosis* (PPD) or soluble *Schistosoma mansoni* egg antigens (SEA) [13, 14]. Using these models, we report the effect of slow release recombinant IL-10 and IL-12 on parameters of leukocyte infiltration, inflammatory macrophage function, and lymphocyte maturation in draining lymph nodes. Our results show that the cytokine treatments have both common and divergent effects in the two models. The data also emphasize the importance of understanding the spectrum of counter-regulatory mechanisms invoked by exogenous cytokine treatment.

Materials and methods

Animals

Female, CBA/J (The Jackson Laboratories, Bar Harbor, ME, USA) mice were used in all experiments. Mice were maintained under specific pathogen-free conditions and provided with food and water ad libitum.

Sensitization and granuloma induction

Pulmonary granulomas with predominantly type 1 or type 2 cytokine involvement were generated as previously described [13]. Briefly, mice were sensitized by i.p. injection of 4 mg (wet weight) of live Bacille-Calmette-Guerin (BCG) strain of *M. bovis* (Organon Technika, Durham, NC, USA) or 3000 *S. mansoni* eggs suspended in 0.5 ml phosphate buffered saline (PBS). Fourteen to 16 days later BCG and egg sensitized mice were respectively challenged by i.v. with 6000 Sepharose 4B beads (in 0.5 ml PBS) covalently coupled with purified protein derivative (PPD) of *M. tuberculosis* or soluble schistosome egg antigens (SEA) obtained from the World Health Organization, Geneva, Switzerland.

Cytokines

Recombinant murine IL-12 was kindly provided by Genetics Institute, Cambridge, MA, USA. Recombinant human IL-10 was kindly provided by Amgen, Thousand Oaks, CA, USA.

In vivo cytokine treatment by osmotic pump implantation

Two days before time of bead challenge mice were subjected to ketamine anesthesia, the abdomen was prepped and cleaned with 70% alcohol. A 0.8 cm incision was made in the skin and peritoneum through which was implanted an osmotic pump (Alza Corp, Palo Alto, CA, USA) containing 100 µl of IL-10 or IL-12 solution with concentration adjusted to achieve a constant infusion of 50 µg/kg/day (about 40 ng/mouse/h); this resulted in steady state serum cytokine concentrations of 3.2 ± 0.9 ng/ml. Control pumps contained vehicle, PBS. After two days rest, the mice were challenged I.V. with antigen-coated beads. Four days after challenge, lungs and lymph nodes were excised and cultures prepared as described below. In all experiments, samples of lungs were fixed in 10% buffered formalin for morphometric analysis.

Granuloma, granuloma macrophage and lymph node culture

Groups of mice were killed 4 days after bead embolization, the expected time of maximum lesion size. Following perfusion with cold RPMI, lungs excluding trachea and major bronchi were excised, placed in cold RPMI medium then homogenized in a Waring blender with a narrow-bottom stainless steel cup. Intact GR were collected over a sterile stainless steel mesh (#100) and rinsed with cold RPMI. Granuloma macrophages were obtained by digestion of GR in a membrane sterilized solution of RPMI-1640 medium (JRH Biosciences, Lenexa, KS) containing 10% fetal bovine serum (Intergen, Purchase, NY), 10 mM glutamine, and 100 mg/ml streptomycin and 100 U/ml penicillin (RPMI-FBS) containing 1000 U/ml type IV collagenase (Sigma, St. Louis, MO, USA). Following 30 min incubation in a 37° shaker water bath at 120 cycles/min, the digest was passed through a stainless steel mesh (#100) and washed four times in RPMI-FBS. Macrophages were isolated by 2 h adherence and monolayers were washed twice then immediately cultured in the presence or absence of 1 µg/ml endotoxin in a 37 °C incubator with a 5% CO_2, humidified atmosphere. Endotoxin stimulation was best for evaluation of macrophage IL-12 and TNF producing capacity. Supernatants were collected at 48 h and stored at −45 °C. Adherent cells on dishes were stained and the total number of adherent macrophages were counted in order to normalize cytokine production. Adherent cells were >90% macrophages based upon morphology and nonspecific esterase staining. For differential counting, duplicate cytospin preparations of digested granulomas were prepared and stained with Wright's stain.

Mediastinal lymph notes were collected at the time of lung harvest and teased into single cell suspension. After washing, the cells were cultured in RPMI FBS at 5×10^6/ml in the presence or absence of 5 µg/ml PPD or SEA, then cultured as above for 36 h. Supernatants were collected by centrifugation and stored at −45 °C.

Cytokine measurement

Interleukins 2, 4, 5, 10, TNF and MCP-1 were measured by ELISA using commercially available reagents (Pharmingen, San Diego, CA, USA); sensitivities were at least 50 pg/ml. Commercially available recombinant murine cytokines served as standards in all assays (Genzyme, Cambridge, MA and Preprotech Inc., Rocky Hill, NJ, USA). IL-12 was measured by sandwich ELISA as described [15] and was sensitive to 50 pg/ml. Interferon-γ was measured by ELISA as described [16] using a capture antibody derived from the XMG-6 clone, kindly provided by Dr. F. Finkelman, NIH; sensitivity was to 50 pg/ml.

Statistics

The Student's t-test was used to compare groups. Values of $p > 0.05$ were considered to indicate lack of significance.

Results

Effect of IL-10 and IL-12 treatment on granuloma size and composition

We initially examined the gross effect of IL-10 and IL-12 infusion on the overall size of PPD (type 1) and SEA (type 2) pulmonary granulomas (GR). Figure 1 shows that IL-10 infusion (50 µg/kg/d) did not alter the size of either type of

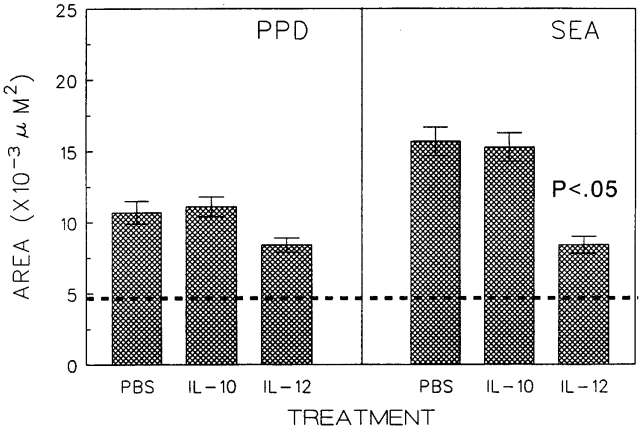

Fig. 1. Effect of constant IL-10 or IL-12 infusion on the size of PPD (type 1)- and SEA (type 2) pulmonary granulomas. CBA mice were presensitized, implanted with control or cytokine infusion pumps then challenged with PPD- or SEA-beads as described in Materials and Methods. On day 4 of granuloma formation, lungs were excised, fixed, and paraffin-embedded for histologic examination. Cross-sectional areas of individual granulomas were determined by computerized morphometric analysis. Bars are mean granuloma areas + SEM of a representative experiment (4–5 mice per group in each experiment). A minimum of three separate experiments were performed.

Table 1. Effect of IL-10 and IL-12 infusion on cellular composition of PPD (type 1) and SEA (type 2)-bead lesions.

Lesion type and treatment	Lymphocytes	Large mononuclears	Eosinophils	Neutrophils
PPD-bead (type 1)				
PBS	56 ± 4	34 ± 5	1 ± 1	9 ± 3
IL-10	59 ± 5	38 ± 4	0 ± 0	2 ± 2*
IL-12	50 ± 6	43 ± 8	2 ± 1	5 ± 2
SEA-bead (type 2)				
PBS	38 ± 6	32 ± 6	28 ± 7	2 ± 1
IL-10	40 ± 6	33 ± 2	25 ± 6	2 ± 1
IL-12	57 ± 8*	39 ± 9	1 ± 1*	3 ± 2

Values are mean percentages ± SD derived from three separate experiments. Differentials were determined from Wright cytospin preparations of cells from enzymatically dispersed granulomas. *$p < 0.05$.

lesion. It should be noted that an effect was likewise not observed at doses of 100 μg/kg/d (data not shown). In contrast, IL-12 infusion caused a pronounced reduction in the size of type 2 lesions (range 50–70% among three separate experiments) but only a minimal reduction in type 1 lesions (range 5–16% among three separate experiments). It should also be mentioned that no significant inflammatory reaction was observed within the peritoneum in response to the pumps or the infused cytokines.

In order to determine possible effects of GR composition, differential analysis was performed on dispersed GR cells. As shown in Table 1, type 1, PPD-bead lesions were composed primarily of lymphocytes, mononuclear phagocytes, and neutrophils, whereas type 2, SEA-bead lesions have a significant component of eosinophils. Interleukin 10 treatment reduced the neutrophil component of the type 1 lesion while the type 2 lesion was unaffected. In contrast, IL-12 treatment did not significantly affect the type 1 lesion but it virtually eliminated the eosinophil component of type 2 lesions, resulting in a composition similar to the type 1 lesion.

Effect of IL-10 and IL-12 treatment on granuloma macrophage cytokine producing capacity

We previously reported that macrophages isolated from PPD and SEA bead GR display different patterns of cytokine production. Reflecting the differential participation of Th1 and Th2 cells [13, 14]. As shown in Figure 2A, GR macrophages of type 1 lesions display strong IL-12 and TNF but weak MCP-1 production, whereas those of type 2 lesions show weak IL-12 and TNF but strong MCP-1 production. Levels of IL-10 were comparable in macrophage cultures of both types of lesions.

Infusion of IL-10 had no effect on type 2 GR macrophages but caused a reduction of all cytokines produced by type 1 GR macrophages (Fig. 2B). Infusions of IL-12 likewise had different effects on macrophages from the two types of lesions (Fig. 2C). Interestingly, IL-12 had the common effect of reducing MCP-1 production by macrophages of both lesion types but otherwise the effects differed. While TNF and IL-10 were unaffected, extrinsic IL-12 seemed to impair intrinsic IL-12 producing capacity in the type 1 lesion. In contrast, extrinsic IL-12 augmented intrinsic IL-12 producing capacity as well as IL-10 and TNF production by type 2 lesion macrophages. These diverse effects on GR macrophages were likely the result of influences on both macrophage function and T cell-derived cytokine production.

Effect of IL-10 and IL-12 treatment on serum IFN-γ levels

IL-10 and IL-12 are known to respectively suppress and augment IFN-γ production in vitro. In order to assess this potential activity in vivo, levels of IFN-γ were measured in the sera of cytokine treated mice. As shown in Table 2, IFN-γ was detectable in the sera of PPD-bead challenged mice but was undetected in the SEA-bead challenged mice. Unexpectedly, IL-10 treatment did not decrease but rather tended to increase levels of IFN-γ in the type 1 response. As would be predicted, IL-12 profoundly augmented serum IFN-γ levels in both PPD and SEA bead challenged mice.

Effect of IL-12 and IL-10 treatment on lymph node cytokine profiles

In order to determine the regional effect of cytokine infusion on lymphoid cell maturation, mediastinal lymph nodes cells were collected from granuloma bearing mice and assessed for antigen-induced cytokine profiles. As shown in Figure 3 (hatched bars), the type 1 PPD response was dominated by IFN-γ and IL-12 production. Treatment with IL-10 reduced IL-12 in a manner consistent with our previously published observations (Fig. 3A) [15]. Surprisingly, IL-10 augmented IFN-γ levels (Fig. 3A), an effect seemingly inconsistent with its known ability to inhibit IFN-γ synthesis. This paradoxical result was consistent with the unexpected serum elevation of IFN-γ and indicated that there were cellular sources if IFN-γ resistant to the effects of IL-10.

Table 2. Serum γ-interferon levels following IL-10 and IL-12 infusion in PPD- and SEA-bead challenged mice.

Lesion type	Treatment and IFN-γ levels (ng/ml)		
	PBS	IL-10	IL-12
PPD-bead (type 1)	0.05 ± 0.06	0.13 ± 0.06	8.5 ± 2.8*
SEA-bead (type 2)	< 0.05	< 0.05	8.2 ± 1.4*

Values are means ± SD. Serum was collected at the time of sacrifice, day 4, and assayed for IFN-γ by ELISA. Levels in untreated, naive mice were less than lower limit of assay. *$p < 0.005$.

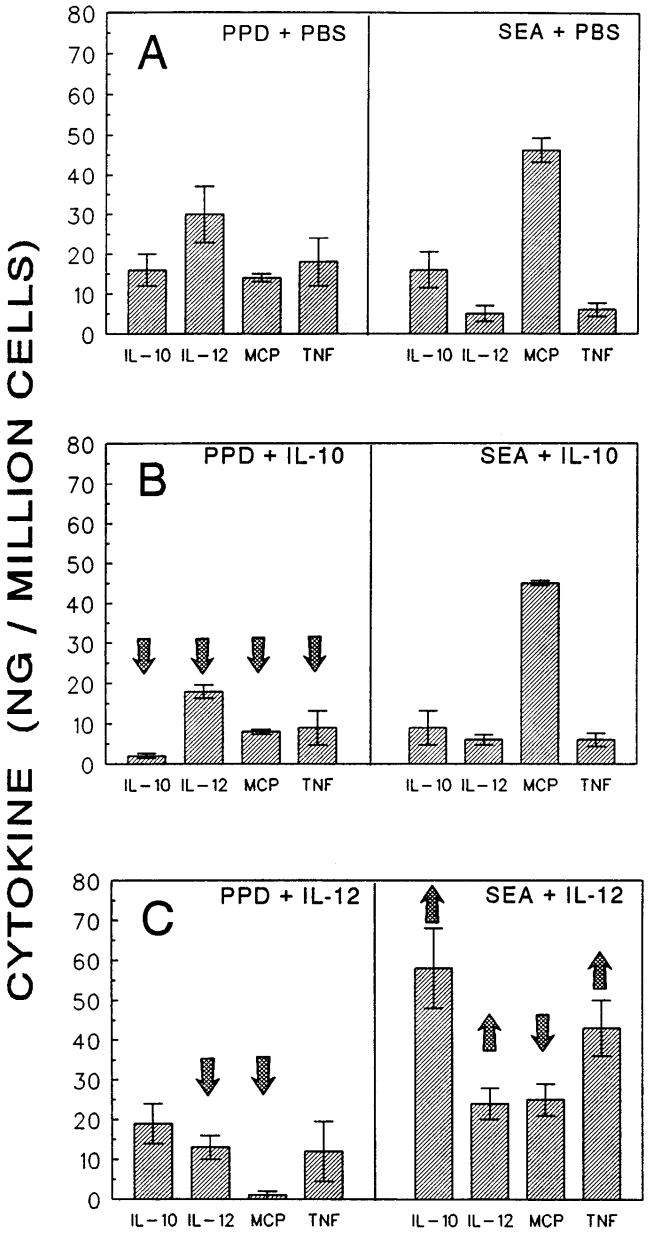

Fig. 2. Effect of constant IL-10 or IL-12 infusion on the production of IL-10, IL-12, MCP-1 and TNF by isolated PPD (type 1)- and SEA (type 2)-bead granuloma macrophages. (A) Cytokine profiles of GR macrophages from mice with control PBS pumps. Patterns are not significantly different from untreated mice. (B) Mice with IL-10 pumps. (C) Mice with IL-12 pumps. Bars are means ± SEM and show levels of spontaneously released IL-10 and MCP-1 and endotoxin-elicited IL-12 with TNF. Arrows indicate the direction of statistically significant changes from mice with PBS pumps derived from three separate experiments. All levels are normalized to 1×10^6 macrophages.

As expected, IL-12 treatment profoundly augmented IFN-γ and reduced IL-2 producing cells (Fig. 3B), likely reflecting a shift from IL-2 producing precursors to mature Th1 cells [17]. Since IL-2 levels were variable between experiments, the latter effect was most evident in those experiments with a larger pool of IL-2 producing cells. Surprisingly, similar to our findings with IL-10, exogenous IL-12 suppressed intrinsic IL-12 production, suggesting a feedback regulatory loop.

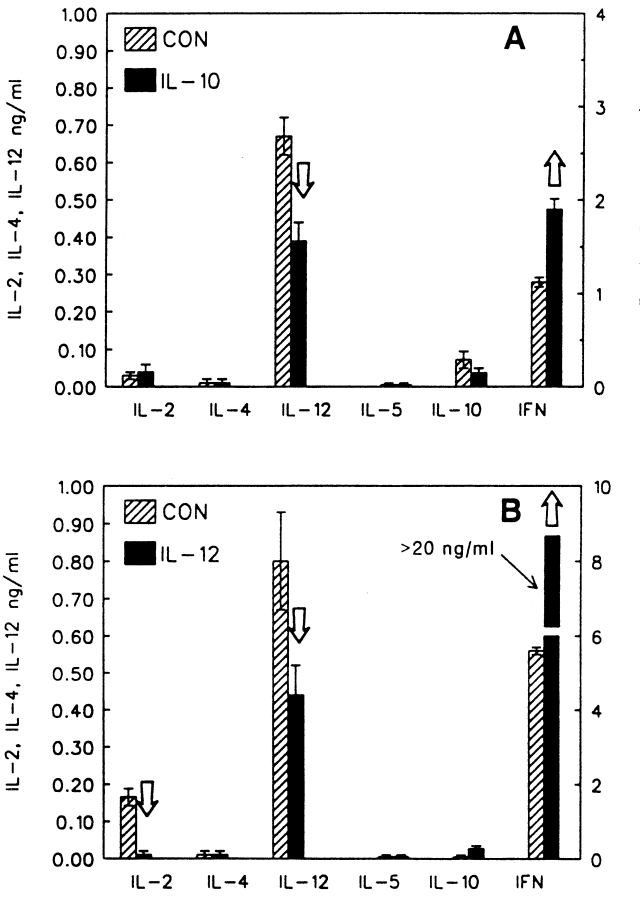

Fig. 3. Effect of constant IL-10 or IL-12 infusion on cytokine profiles of draining lymph node cell cultures from mice with PPD (type 1) pulmonary granulomas. Mediastinal lymph nodes of control or cytokine treated mice (4–5 mice/group) with 4 day, type 1 PPD GR were pooled, dispersed and cultured in the presence or absence of PPD (5 μg/ml) for 36 h. Supernatants were then assayed for the cytokines indicated. (A) IL-10 treatment study; (B) IL-12 treatment study. Bars are means ± SEM of three determinations from a representative experiment. Three separate experiments were performed. Left scale applies to IL-2, IL-4 and IL-12 and right scale to IL-5, IL-10, and IFN-γ. Arrows indicate direction of significant changes.

The type 2 SEA response was dominated by IL-4, IL-5 and IL-10 production with weak IFN-γ and negligible IL-12 production (Fig. 4). Unexpectedly, IL-10 reduced expression of all Th2 cytokines as well as IFN-γ (Fig. 4A). In contrast, IL-2 levels were increased, suggesting a shift to less differentiated T cells. As in the type 1 response, IL-12 infusion augmented IFN-γ and reduced IL-2 producing cells (Fig. 4B). In addition, all Th2-related cytokines were profoundly abrogated. These changes were consistent with abolition of Th2 expression and conversion to a Th1-like response.

Discussion

There is substantial evidence indicating that IL-10 and IL-12 are potent immunoregulatory cytokines and consequently they are suspected to have potential therapeutic use. Specifically, IL-10 is thought to have anti-inflammatory properties by virtue of its ability to suppress the expression

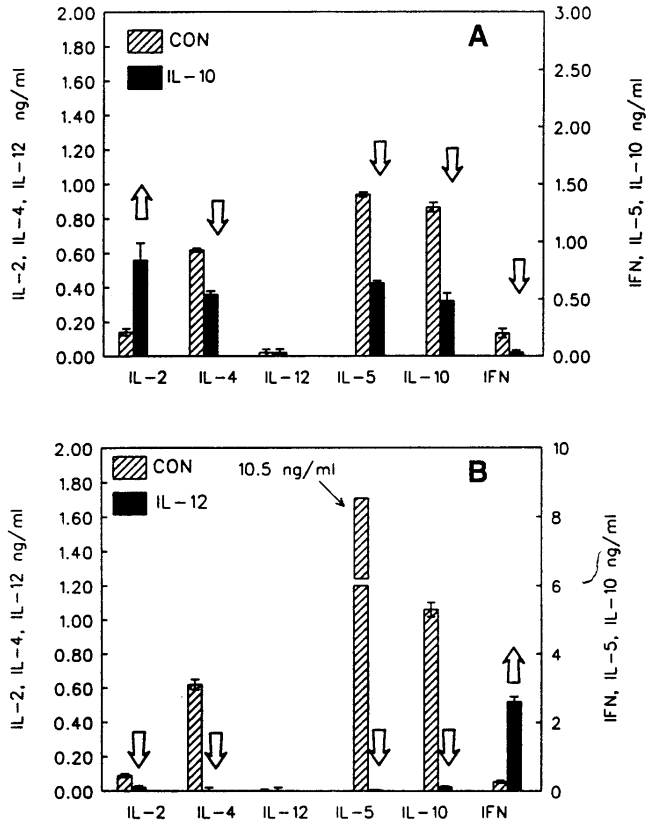

Fig. 4. Effect of constant IL-10 or IL-12 infusion on cytokine profiles of draining lymph node cell cultures from mice with SEA (type 2) pulmonary granulomas. Mediastinal lymph nodes of control or cytokine treated mice (4–5 mice/group) with 4 day, type 2 SEA GR were pooled, dispersed and cultured in the presence or absence of SEA (5 μg/ml) for 36 h. Supernates were then assayed for the cytokines indicated. (A) IL-10 treatment study; (B) IL-12 treatment study. Bars are means ± SEM of three determinations from a representative experiment. Left scale applies to IL-2, IL-4 and IL-12 and right scale to IL-5, IL-10, and IFN-γ. Arrows indicate direction of significant changes.

of a number of cytokines such as IFN-γ, TNF and IL-1 [3–5]. Conversely, IL-12 has the ability to enhance IFN-γ and is thought to be proinflammatory [2, 7]. A variety of animal models of human disease have been employed to examine the effects of manipulating IL-10 and IL-12 in vivo. For example, IL-10 treatment caused suppression of inflammation in a model of viral keratitis [18] yet depletion of IL-10 abrogated inflammation in a model of murine lupus erythematosus [19]. Treatment with IL-12 promoted resistance to *Mycobacteria* [20], *Cryptococcus* [21], *Leishmania* [22] and *Cryptosporidia* [23], but impaired responses to the intestinal parasite, *Nippostrongylus brasiliensis* [24]. While these studies appear sometimes conflicting, they likely reflect effects on different host immune mechanisms that involve either Th1- or Th2-related cytokines. In addition, unexpected findings may arise if cytokine manipulation disrupts or invokes unknown regulatory circuits. In order to help clarify the potential effects of IL-10 and IL-12 immunotherapy, the present study employed standardized models of pulmonary granulomatous inflammation that are mediated by predominantly Th1- (type 1) or Th2- (type 2)-related cytokines [13, 14]. Unlike previous studies we used constant IL-10 or IL-12 infusion and performed simultaneous comparisons of local cellular recruitment, GR macrophage function and regional lymphoid tissue responses. Our results not only support a number of previously published observations but provide novel information regarding the local and regional effects of exogenous cytokine treatment during Th1 or Th2 dominated immune responses. The following discussion will summarize these findings and their implications.

Infusion of IL-10 had no effect on the gross size of either type 1 or type 2 GRs as determined by morphometry. This finding is consistent with that of Powrie et al. who found that IL-10 alone had no effect on the T cell-mediated inflammatory response to *Leishmania major* [8]. That study showed that inhibition of T cell-mediated leukocytic infiltration required combined IL-10 and IL-4 treatment. Despite having no effect on lesion size, our study clearly demonstrated that IL-10 had differing effects on GR cell composition and macrophage function. Neutrophil content was abrogated in type 1 GR which may be related to the broad reduction in macrophage cytokine producing capacity with associated reduction in leukocyte recruitment factors. Similarly, IL-10 may have direct inhibitory effects on neutrophils as reported by Wang et al. [25]. In contrast, type 2 GR macrophages were resistant to extrinsic IL-10 and GR cell composition was unaffected. This may be related to the likelihood that cells of type 2 lesions are already exposed to intrinsic IL-10 in the Th2-dominant immune environment. For example, we recently reported that intrinsic IL-10 is in part responsible for the impaired IL-12 producing capacity of type 2 GR macrophages [15]. Similarly, Villaneuva et al. showed that schistosome egg GR macrophages were subject to intrinsic IL-10-mediated down-regulation of B-7 costimulatory molecules [26].

In the draining lymphoid tissue, IL-10 infusion caused a paradoxical augmentation of IFN-γ production seemingly inconsistent with its known Th1 inhibitory effects in vitro [3–5]. Only speculative explanations can be offered at this time. Perhaps, infused IL-10 may promote tachyphylaxis, possibly via receptor desensitization, causing resistance to IL-10 mediated suppression. Alternatively, IL-10 may inhibit production of other factors that impair IFN-γ producing cells. Interestingly, the type 1 GR macrophages were still subject to deactivation by exogenous IL-10, indicating a differential sensitivity or adaptability of local and regional responses. The finding that IL-10 caused macrophage deactivation despite the presence of IFN-γ is fully consistent with studies of Fiorentino et al. [4].

Surprisingly, IL-10 inhibited the production of Th2-related cytokines during the type 2 response seemingly inconsistent with the in vitro studies of Fiorentino et al. [3]. Unlike type 2 GR macrophages, draining lymphoid cells were not resistant to IL-10 treatment. The inhibitory effect of IL-10 on Th2 cytokine profiles may be at the level of antigen-presenting cells. In vitro studies of Kawamura and Furue showed that IL-10 selectively downregulates the expression of B7-2 [27], a costimulatory membrane antigen thought to promote Th2 cell differentiation [17, 28]. Based on this observation, IL-10 might be predicted to impair Th2 cell maturation as our results indicate. Alternatively, as noted above, exogenous IL-10 may induce a tachyphylactic response. We have previously shown that the Th2 profile

in lymphoid tissues of SEA-bead challenged mice is in part dependent on endogenous IL-10 [14]. Constant IL-10 infusion may induce resistance, indirectly eliminating the Th2 supportive function of IL-10. Regardless of the underlying mechanism, this finding is of particular importance since it suggests that while IL-10 treatment may not block Th2 effector function, it may block Th2 cell maturation and expansion within lymphoid tissues, thereby degrading subsequent responses. Consequently, IL-10 may be useful for treatment of Th2 based inflammatory diseases.

In addition to corroborating previously reported observations, our studies employing IL-12 infusions provide a number of novel observations. As would be predicted, IL-12 promoted the expression of IFN-γ producing cells in the regional lymphoid tissue during both types 1 and 2 GR formation. Furthermore, in accord with studies of Wynn et al. [11] and Oswald et al. [12], IL-12 treatment profoundly abrogated the eosinophil-rich GR response to schistosomal egg antigens as well as Th2 cell expression in regional lymphoid tissue. Despite the common effect of promoting IFN-γ production, our studies revealed subtle differences in responses to IL-12 that suggest the presence of a complex network of cytokine regulation as discussed below.

Extrinsic IL-12 suppressed intrinsic IL-12 producing capacity in the type 1 response but augmented it in the type 2 response. We have previously shown that IL-12 is down-regulated during the type 2 GR response by IL-4/IL-10 mediated suppression [15]. Presumably, extrinsic IL-12 largely eradicates the Th2 influence thereby relieving this suppressive effect. In contrast, the type 1 GR response is characterized by augmented IL-12 producing capacity. Apparently, in this environment extrinsic IL-12 acts to inhibit intrinsic IL-12 production, overcoming the known stimulatory effect of IFN-γ [29]. Thus, IL-12 can potentially act in a feedback manner to limit its own production.

Differential regulatory effects of IL-12 were also apparent with regard to GR macrophage IL-10 and TNF production. The GR macrophages of type 1 lesions showed no significant changes, whereas those of type 2 GR showed augmentations of these cytokines. As with IL-12, the enhanced TNF producing capacity may be due to lessened IL-4/IL-10-mediated suppression [30] and augmented IFN-γ-mediated stimulation [31, 32]. The enhanced IL-10 expression is especially intriguing. In previous studies IL-12 injections enhanced IL-10 mRNA levels in lungs with schistosome egg GR [11] and in livers of otherwise untreated mice [33]. It is not clear if this is a direct or indirect effect. In any case, these findings suggest another regulatory loop in which IL-12 induces IL-10, a potential cross-regulatory molecule. The apparent resistance of type 1 GR macrophages to this inducing effect is likewise intriguing and further studies will be needed to determine the underlying mechanism.

The abrogation of GR macrophage derived monocyte chemotactic protein-1 (MCP-1) production by IL-12 infusion was another novel finding. Inhibition was observed in both types of lesions. At present, it is not clear if this is a direct or indirect effect of IL-12. In addition to being produced at higher levels by type 2 GR macrophages, depletion studies indicate that MCP-1 contributes more to type 2 than to type 1 inflammation [34, 35]. Furthermore, we have recently demonstrated that intrinsic MCP-1 supports Th2 cell expression and directly inhibits IL-12 production [36]. Taken together, these data suggest that MCP-1 and IL-12 are cross-regulatory, extending the known functional roles of these molecules.

The results of the present study emphasize the need to understand the complexity of cytokine regulatory networks as related to site (i.e. local, regional and systemic) and immune status. Such knowledge will be essential to the design of rational cytokine-based immunotherapy.

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