Inflammation, Vol. 27, No. 4, August 2003 (© 2003)

Impairment of Leukocyte Trafficking in a Murine Pleuritis Model by IL-4 and IL-10

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Abstract—We have characterized leukocyte migration to the pleural cavity in a methylated-BSA (mBSA)-induced model of murine delayed-type hypersensitivity and evaluated the ability of IL-4 and IL-10 to modulate this response. Neutrophils, macrophages, T cells, and dendritic cells migrated to the pleural cavity in a time-dependent fashion following direct intrapleural antigen challenge, with neutrophils comprising the majority of exudate leukocytes in the cavity within the first 24 h and the number of mononuclear cells increasing at later times. Real-time quantitative PCR analysis of infiltrating leukocytes revealed a marked elevation of steady-state mRNA levels of IL-1β and TNFα and the chemokines KC, MIP-2, CXCL9, CXCL10, CXCL11, CCL2, CCL3, and CCL4 at 6 h postchallenge, which diminished over time. In contrast, γIFN mRNA levels were maximal at 24 h and CCL5 expression was sustained throughout 72 h. ELISA analysis of pleural exudate fluid revealed significant elevations of KC and CCL2 protein levels at 6 h postantigen challenge and a peak increase in γIFN protein at 24 h, confirming our mRNA observations. Administration of recombinant murine IL-4 or IL-10 prior to challenge significantly blocked cell trafficking to the pleural cavity as well as peak levels of exudate γIFN, with IL-4 being more potent in impairing these responses. IL-4 administration also increased the proportion of naïve T cells in the pleural cavity, as judged by CD62L and CD45RB expression. These results indicate that this *in vivo* model demonstrates a pattern of events associated with Th1-mediated leukocyte trafficking and underscore the potential utility of this *in vivo* model for evaluating therapeutic inhibitors of leukocyte trafficking.

KEY WORDS: chemokine; cytokine; leukocyte trafficking; pleuritis.

INTRODUCTION

The pathogenesis of autoimmune diseases such as rheumatoid arthritis, Crohn's disease, and multiple sclerosis involves the activation of Th1-mediated inflammatory responses, including the production of an array of proinflammatory cytokines and chemokines, which amplify and exacerbate immune responses directed against specific organs or tissues (1–5). Recent efforts have focused on identifying and characterizing the role of a number of these molecules in the development of a range of rodent models of inflammation and autoimmunity, using neutralizing antibodies or knockout mice (reviewed in 1, 6). In many cases, assessment of leukocyte migration to sites of inflammation in these models requires histological or extensive leukocyte purification procedures, rather than direct cell isolation and analysis, to accurately quantitate cell migration to inflamed sites.

Methylated BSA (mBSA) is a versatile protein antigen that stimulates a "tuberculin-like" Th1 delayed type hypersensitivity (DTH) response in mice without evidence of an Arthus reaction (7), and permits the induction of type IV DTH inflammatory responses in the skin (8), joint

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0360-3997/03/0800-0161/0

(9–15), and pleural cavity (16). In the latter case, this response was associated with pronounced leukocyte trafficking to the pleural space and the formation of exudate fluid (16). In the present report, we have refined and further characterized this model by examining the kinetics of the trafficking of leukocyte subsets to the pleural cavity and correlated it to local chemokine and cytokine expression. Moreover, we demonstrate that leukocyte trafficking is profoundly impaired by systemic administration of IL-4 or IL-10. These observations suggest its value for defining critical cytokine- and chemokine-mediated pathways of inflammation.

MATERIALS AND METHODS

Induction of mBSA-Induced Pleuritis

Female CF-1 mice (Charles River Laboratories, Wilmington, MA), at 6–9 weeks of age, were immunized subcutaneously with a 1:1:1 emulsion of mBSA (50 mg/mL in saline), complete Freund's adjuvant (Sigma) and 0.5 mg of *Mycobacterium tuberculosis* dried cell walls (stains C, DT, and PN; Veterinary Laboratory Agency, Surrey, UK). This preparation was carefully emulsified on ice until a stiff frothy mixture was obtained. The consistency of the emulsion was tested periodically during preparation by placing a droplet of emulsion on the surface of ice water. When the droplet remained intact, the emulsion was loaded into glass syringes and delivered by subcutaneous injection near the axillary lymph node in a volume of 0.2 mL. The total amount of antigen each animal received at sensitization was 3.3 mg. Fourteen days later, the mice were anesthetized by inhalation of isofurane vapors, and the mice challenged intrapleurally with 200 μg mBSA in 0.2 mL endotoxin-free saline (Abbott Labs, N. Chicago, IL) using a 26° needle. The intrapleural injection of antigen required that the animal be placed on its side and a small skin incision made at the last rib in line with the forepaw to allow for visualization of the rib cage. The last rib was grasped with a small toothed forcep and lifted, and the needle slid between the last two ribs just past the bevel to deliver the antigen. This procedure minimized the chance of puncturing the lung. Controls typically consisted of mice that received endotoxin-free saline intrapleurally on day 14 (no challenge). In some experiments, leukocyte counts were also evaluated in an additional set of control mice that were subcutaneously immunized with adjuvant without mBSA and challenged on day 14 with mBSA (nonsensitized; see Fig. 1 legend). Immediately after injection, the skin was closed with sterile wound clips (Fisher Scientific, Springfield, NJ). At 6–72 h postantigen challenge, the mice were euthanized and pleural cavity exudate harvested by making a small incision and injecting 1 mL of chilled cell dissociation buffer (GIBCO-BRL, Grand Island, NY) containing 0.1% heparin (Fisher) into the pleural cavity. This buffer significantly reduced cell clumping in the exudate. Care was taken to wash the entire cavity gently using a fine plastic disposable Pasteur pipet and to avoid inducing bleeding into the cavity, which would compromise results. In some experiments, 20 μg of recombinant murine IL-4 or IL-10 (<0.1 EU/mg) were administered subcutaneously in saline twice daily starting 2 days prior to antigen challenge, after preliminary dose–response experiments were performed to establish the maximal effective dose of these cytokines. All animal work was performed in accordance with protocols and guidelines established by our institution's Animal Care and Use Committee.

Pleural Exudate Cell Preparation, FACS Analysis and Quantitation of Cytokine/Chemokine Levels in Exudate Fluids

Pleural exudates were centrifuged at 200 $\times g$ to pellet the leukocytes and exudate volume was measured. Exudate was stored at -20°C for subsequent ELISA analysis (R&D Systems; Minneapolis, MN). Leukocytes were prepared by washing twice in 1 mL Alsevier's solution (Sigma) to minimize clotting and erythrocytes lysed by treatment with ACK buffer (Biosource International, Camarillo, CA). The cells were prepared by fixation in 2.5% glutaraldehyde containing crystal violet dye (Sigma) and enumerated with a hemocytometer. Cell viabilities were routinely >95% prior to fixation, as judged by propidium iodide exclusion and flow cytometric analysis. Fluorochrome-conjugated antibody staining (BD Biosciences, Mountain View, CA) and FACS analysis was performed using a FACSCalibur (BD Biosciences), after confirming that assignment of cell populations correlated with cytological examination, as previously described (17). The number of leukocytes of a given phenotype was obtained by multiplying the absolute number of cells per cavity per mouse by the percentage of cells of a specific phenotype. All data are expressed as the mean $\pm$ SEM of at least five mice per treatment group and represent at least three separate experiments.

Quantitative Real-Time PCR

Pleural exudate leukocytes were collected as described above, centrifuged and resuspended into

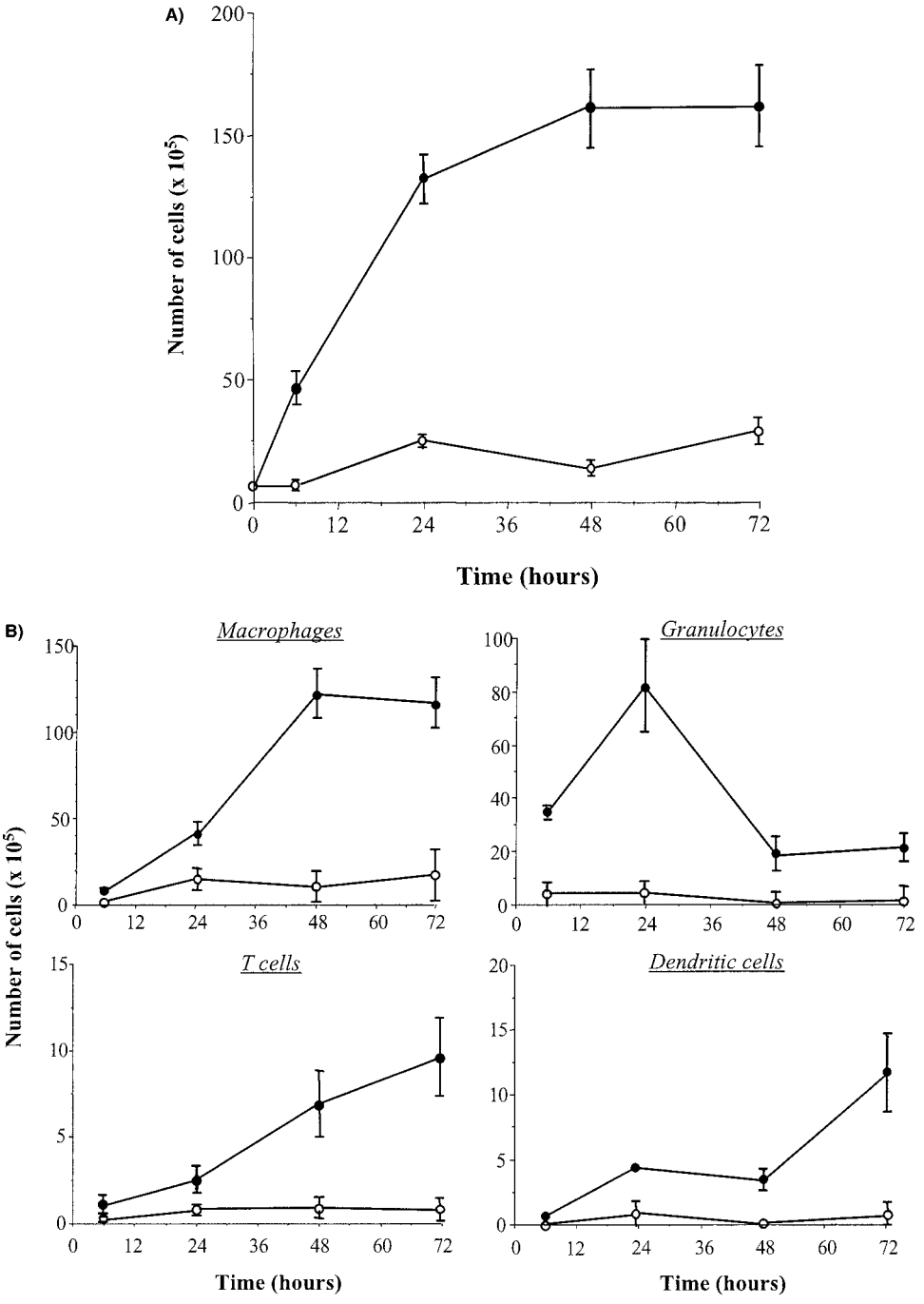


Fig. 1. Leukocyte trafficking to the pleural cavity is time- and cell-type-dependent. A. Timecourse of total leukocyte migration. B. Timecourse of different leukocyte populations. Filled circles, mBSA-challenged mice immunized 2 weeks previously; open circle, no challenge refers to immunized mice that were intrapleurally administered saline. The pleural cavities of nonsensitized mice (no immunization but mBSA challenge) had $13.0 \pm 2.5 \times 10^5$ cells at 24 h. The pleural cavity of normal mice contained $6.2 \pm 0.9 \times 10^5$ cells. In A, differences at all timepoints are statistically significant at $p < 0.001$. In B, differences in macrophage, granulocyte and T cell populations at all timepoints are statistically significant at $p < 0.001$, while differences in the dendritic cell populations are significant at the 24–72-h times at $p < 0.001$.

Table 1. Primer Sets Used for Taqman Analysis of Cytokines and Chemokines

	Forward primer	Reverse primer
Cytokine		
γIFN	CTGGAGGAACTGGCAAAAG	TTCAAAGAGTCTGAGGTAGAAAGAGATAAT
IL-1β	GACGGCACACCCACCCT	AAACCGTTTTTCCATCTTCTTCTTT
IL-4	TCATCGGCATTTTGAACGAG	TTTGGCACATCCATCTCCG
IL-6	ACACATGTTCTCTGGGAAATCGT	AAGTGCATCATCGTTGTTCATACA
IL-10	TTTGAATTCCCTGGGTGAGAA	GCTCCACTGCCTTGCTCTTATT
IL-12 p40	ACAGCACCAGCTTCTTCATCAG	TCTTCAAAGGCTTCATCTGCAA
IL-13	TTGAGGAGCTGAGCAACATCAC	GCGGCCAGGTCCACACT
TGFβ_1	CCCGAAGCGGACTACTATGC	CGAATGTCTGACGTATTGAAGAACA
TNFα	GCCACCACGCTCTTCTGTCT	GGTCTGGGCCATAGAACTGATG
Chemokine		
KC	CCACCCGCTCGCTTCTC	CACTGACAGCGCAGCTCATT
MIP-2	CCTGCCAAGGGTTGACTTCA	TTCTGTCTGGGCGCAGTG
CXCL9	TGCACGATGCTCCTGCA	AGGTCTTTGAGGGATTTGTAGTGG
CXCL10	GACGGGTCCGCTGCAACTG	GCTTCCCTATGGCCCTCATT
CXCL11	CAGGAAGGTCACAGCCATAGC	TTTCTCGATCTCTGCCATTTTG
CCL2	GCTGGAGCATCCACGTGTT	ATCTTGCTGGTGAATGAGTAGCA
CCL3	CCAAGTCTTCTCAGCGCCAT	GAATCTTCCGGCTGTAGGAGAAG
CCL4	TCTGCGTGTCTGCCCTCTC	TGCTGAGAACCCTGGAGCA
CCL5	GCAAGTCTCCAATCTTGCA	CTTCTCTGGGTTGGCACACA
Housekeeping		
Ubiquitin	TGGCTATTAATTATTCGGTCTGCAT	GCAAGTGGCTAGAGTGCAGAGTAA

TriReagent (Molecular Research Center, Cincinnati, OH). RNA was prepared according to the manufacturer's instructions, quantitated using an Agilent 2100 (Agilent Technologies, Palo Alto, CA), and reverse transcribed into cDNA using random hexamers and oligo-dT (GIBCO-BRL). The expression of chemokines and cytokines was determined by real-time quantitative PCR using an ABI 5700 sequence detection system (Applied Biosystems, Foster City, CA). Reactions were performed by adding cDNA (50 ng) and primers (final concentration of 300 nM) to 2× reaction master mix under the following thermal cycling conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of amplification at 95°C for 15 s and 60°C for 1 min for denaturation and extension. The primers used are listed in Table 1. All data was normalized to ubiquitin and expressed as % ubiquitin.

Statistical Analysis

Statistically significant differences between experimental groups were compared using the Student's t test. A value of $p < 0.05$ was considered to be significant.

RESULTS

We assessed the kinetics of leukocyte infiltration into the pleural cavity following antigen sensitization and intrapleural challenge by examining the total number of leukocytes collected from pleural exudate at various times. At 6 h after mBSA challenge, a marked increase in the number of leukocytes in the pleural cavity was evident (Fig. 1A). Cell numbers continued to increase through 48 h postchallenge, and were maintained through 72 h (Fig. 1A). The pleural cavity of mice challenged with saline (no challenge) contained low numbers of cells throughout this time (Fig. 1A). Animals that originally were administered adjuvant without antigen subcutaneously and that received an intrapleural injection of mBSA also demonstrated minimal cell accumulation in the pleural cavity (nonsensitized), as compared to the pleural space obtained from normal mice (Fig. 1 legend). The antigen specificity of this response was demonstrated by the lack of cell trafficking to the pleural space in mice challenged with an irrelevant antigen, ovalbumin (data not shown). These results are consistent with a classical type IV T cell-mediated delayed type hypersensitivity reaction, which requires previous antigen sensitization to induce an immune response upon challenge (7, 9, 16).

To further characterize the induction of leukocyte trafficking to the pleural cavity in response to mBSA challenge, we evaluated the phenotype of the cells accumulating in the pleural space by multiparameter flow cytometry, using a previously described approach (17). As illustrated in Fig. 1B, there was a marked enhancement in granulocyte

trafficking to the pleural cavity observed by 6 h postantigen challenge, which peaked at 24 h before subsiding. These granulocytes routinely were >95% polymorphonuclear leukocytes, as determined by cytological examination, and the majority expressed high levels of Gr-1 (not shown). The number of macrophages increased steadily throughout the first 48 h and was maintained throughout 72-h postchallenge. These responses correlated with time-dependent changes in the proportion of these leukocyte subsets as well, with the percentage of granulocytes at 24 h typically in the range of 60–80% of leukocytes and decreasing thereafter, while the proportion of macrophages peaked at 48 h at 70–80%. An increase in the number of T cells was also evident throughout 72-h post-mBSA challenge, although this number was consistently less than the number of macrophages (Fig. 1B) and typically comprised 5–10% of the total leukocytes. The T cell population consisted of similar numbers of CD4 and CD8 cells and appeared to be activated, as judged by the proportion of cells with increased forward scatter properties, enhanced expression of CD44 and CD162, and reduced levels of CD62L and CD45RB compared to splenic and lymph node T cells. Similarly, $CD11c^+$ dendritic cells tended to accumulate over time at levels similar to that of the T cells (Fig. 1B). In contrast, unchallenged (Fig. 1B) and nonsensitized (not shown) animals demonstrated much smaller increases in each of the leukocyte populations migrating into the pleural cavity. Overall, these results illustrate a highly coordinated cellular kinetic response to antigen challenge in previously immunized mice.

We further evaluated the induction of this inflammatory pleuritis by investigating the expression of cytokines and chemokines by leukocytes harvested from the pleural cavity, using quantitative real-time PCR. As illustrated in Fig. 2, we observed a time-dependent expression pattern of many of these genes compared to nonchallenged animals. In particular, high expression levels of the cytokines IL-1β and TNFα (Fig. 2A) and the chemokines KC, MIP-2, CXCL9, CXCL10, CXCL11, CCL2, CCL3, and CCL4 (Fig. 2B) were seen at 6 h, which diminished with time. Lower but significant levels of IL-6 and IL-12 p40 were seen at 6 h, which also were reduced with time (Fig. 2A). In contrast, enhanced expression of γIFN, IL-4, IL-10, IL-13 (Fig. 2A), and CCL5 (Fig. 2B) was maintained in a more prolonged fashion in challenged mice. TGFβ was also highly expressed at 6 h relative to nonchallenged mice, but was expressed at similar levels to the nonchallenged animals at later times (Fig. 2A). Qualitatively similar results were observed by Taqman analysis of lung samples from these animals as well (data not shown). These data suggest the time-dependent involvement of cytokines and chemokines in the induction and amplification of this inflammatory response.

We extended our Taqman observations by quantifying the protein expression of a subset of these cytokines and chemokine in the pleural exudates of challenged and nonchallenged mice. Consistent with their mRNA levels, IL-1β, KC, and CCL2 protein were greatest at 6 h postchallenge and decreased with time, with much lower levels measured in nonchallenged animals, and γIFN levels were maximal at 24 h (Fig. 3). CCL5 protein levels were detectable in challenged animals throughout 72 h, also consistent with our observations made by Taqman analysis. The kinetics of MIP-2 protein levels in exudate fluid followed that of KC, but were consistently lower, while TNFα, IL-4, IL-10, IL-12 p40, and IL-13 levels were low or undetectable throughout the timecourse examined (data not shown). Surprisingly, TGFβ protein was highest in the exudate of nonchallenged animals at 6 h and then diminished after this time, while TGFβ levels were maintained in challenged animals throughout the timecourse examined (Fig. 3).

Because of the marked anti-inflammatory activities of the cytokines IL-4 and IL-10 in a wide variety of Th1-like immune responses *in vivo* (18, 19), we evaluated their ability to modulate leukocyte trafficking response in this model. IL-4 exerted a powerful anti-inflammatory effect in this model, with a pronounced inhibition of total leukocyte trafficking through 72-h postantigen challenge (Fig. 4A). In comparison, IL-10 was partially effective in blocking this response (Fig. 4A). The efficacy of IL-4 included a pronounced inhibition of the migration of granulocytes, macrophages, T cells, and dendritic cells, while IL-10 treatment had a more modest effect on the trafficking of each of these cell types (Fig. 4B).

Additional characterization of the anti-inflammatory activity of IL-4 and IL-10 in this model demonstrated that both cytokines significantly ablated the increase in γIFN protein levels at 24-h postchallenge, while IL-10 increased local γIFN levels at the 6-h timepoint (Fig. 4C). IL-4 and IL-10 administration also resulted in a 25–35% inhibition of the protein levels KC, CCL2, and IL-1β at 6 h, although no alteration of protein levels of Th2 cytokines such as IL-4, IL-5, and IL-10 in pleural exudate was seen (data not shown). Cytokine and chemokine mRNA expression demonstrated only occasional, modest ($\leq$2-fold) reductions in IL-4-treated animals (not shown). These data do not point to an obvious mechanism for the differences between the efficacies of IL-4 and IL-10 in this model,

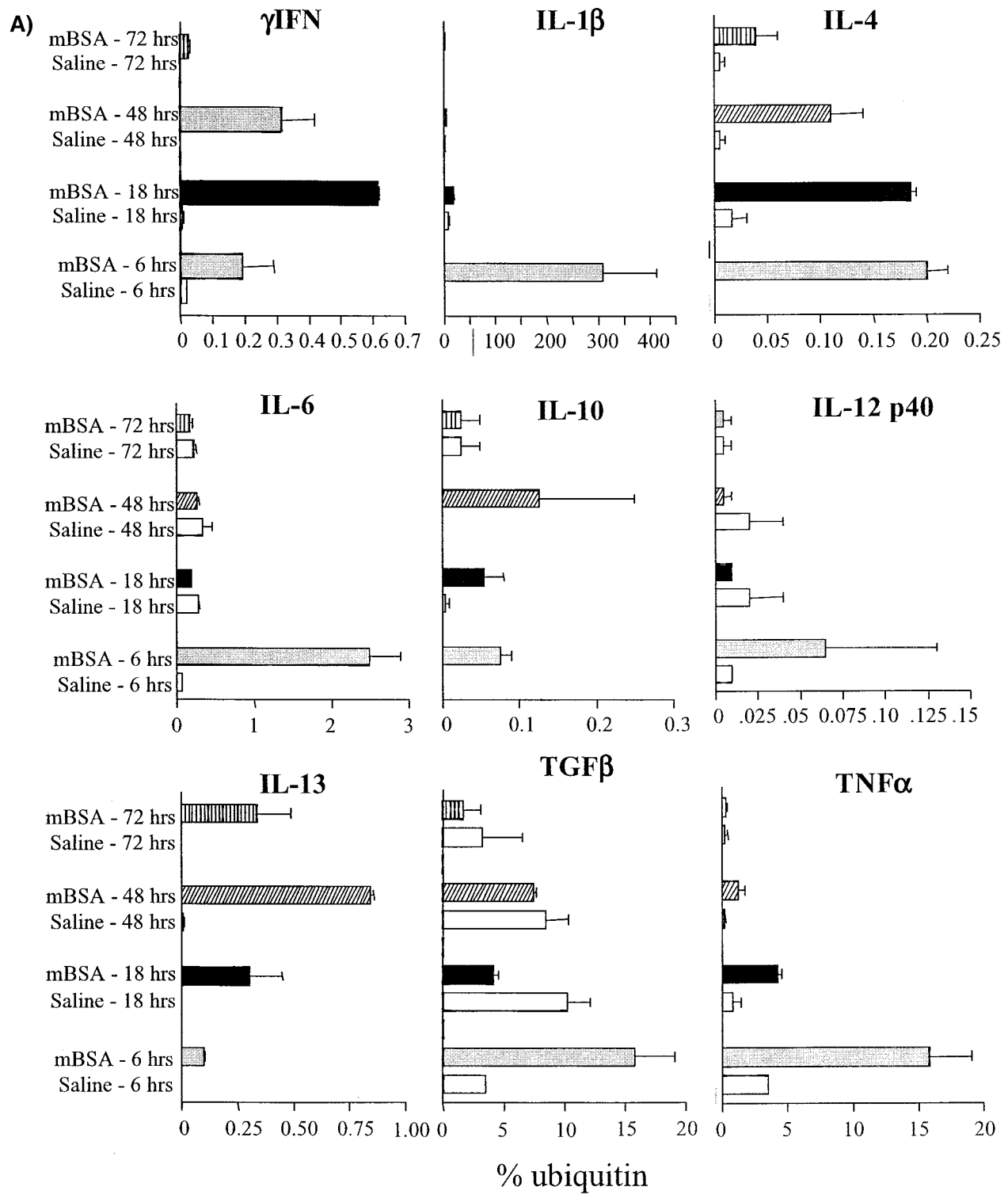
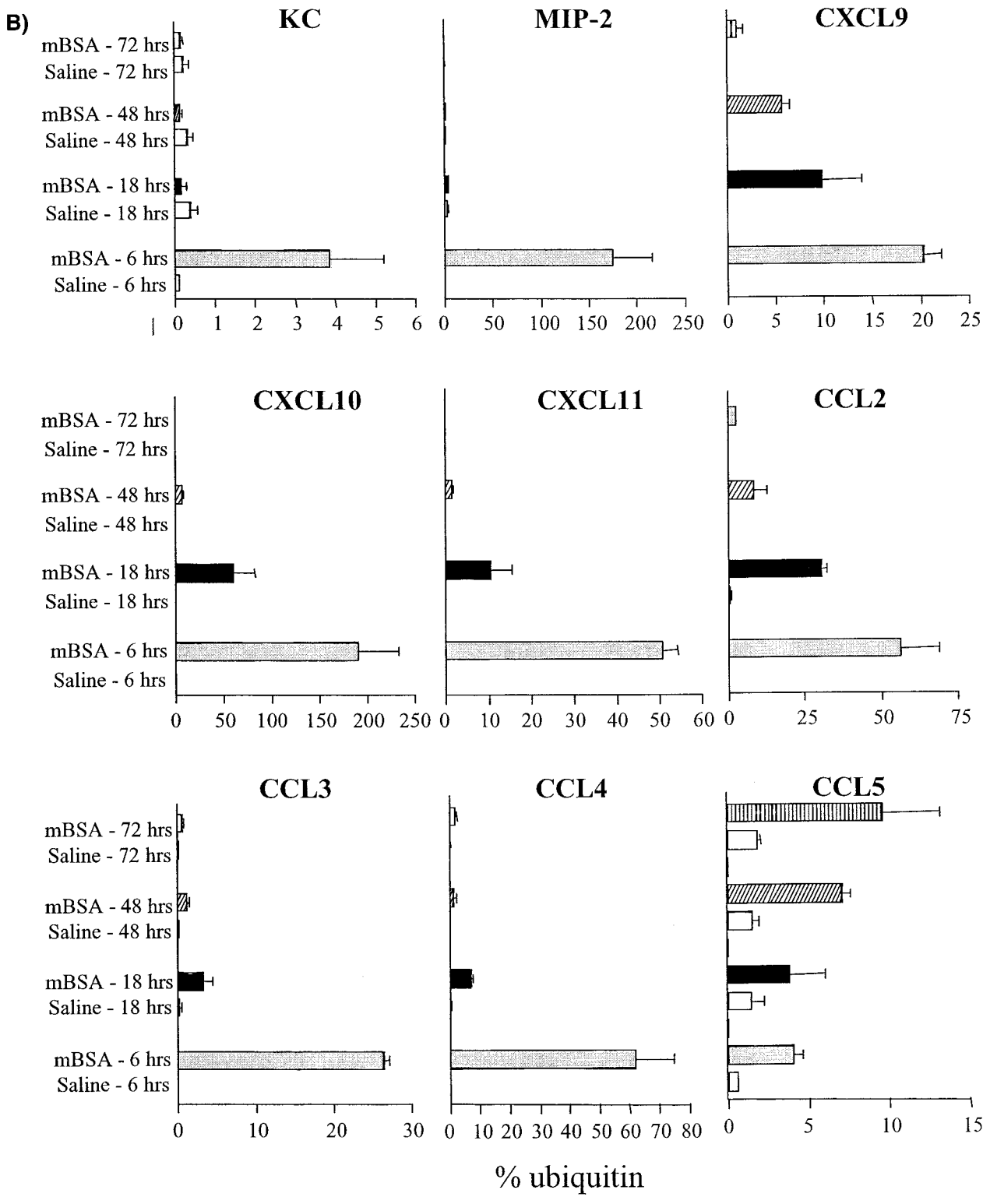


Fig. 2. Real-time PCR analysis of cytokine and chemokine mRNA expression by pleural exudate leukocytes. A Cytokine expression. B. Chemokine expression.



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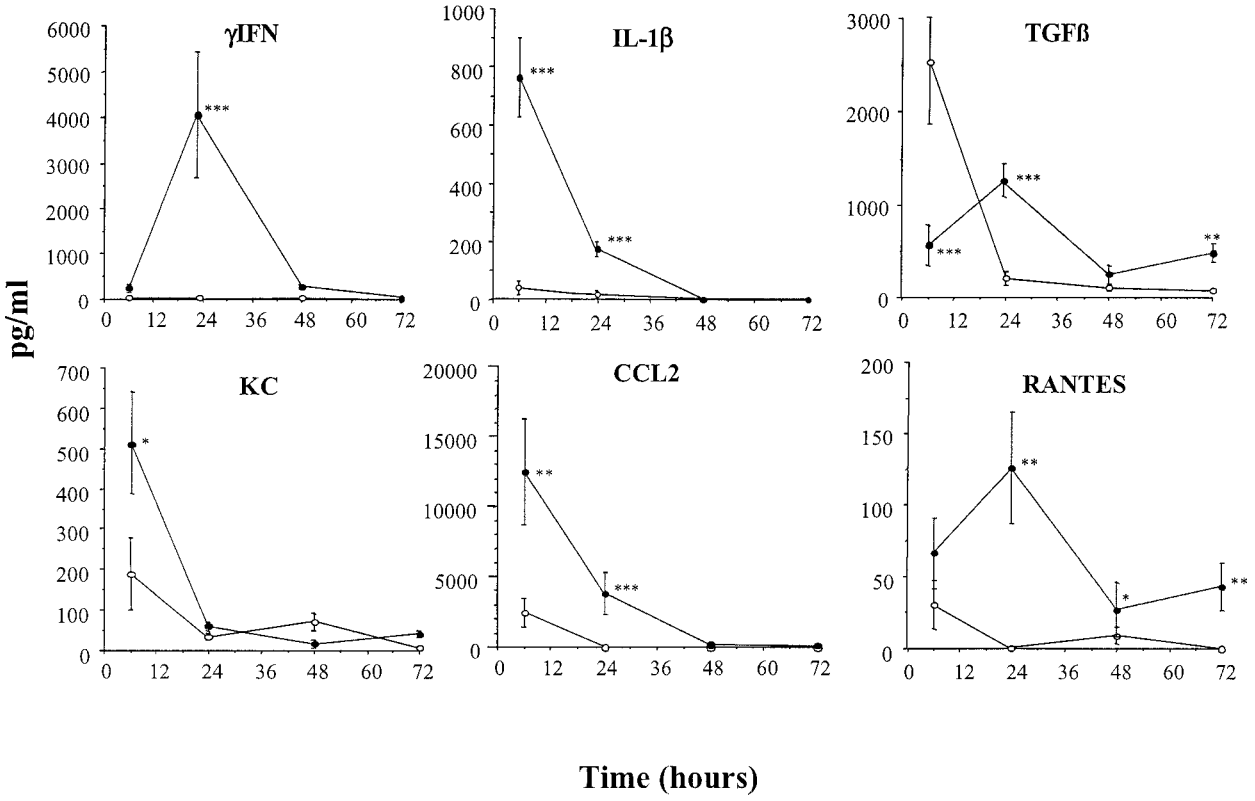

Fig. 3. Evaluation of protein levels of selected cytokines and chemokines in pleural exudate. ELISA analysis of pleural exudates was performed as described in Materials and Methods section. Open circles, no challenge; filled circles, mBSA-challenged animals. Statistically significant differences between groups are as indicated: $^{*}p < 0.05$; $^{**}p < 0.01$, $^{***}p < 0.001$.

although the elevation of γIFN by IL-10 treatment at 6 h after antigen challenge may contribute to its more modest activity.

Differential expression of the surface markers L-selectin (CD62L) and CD45RB has been reported to distinguish naive ($CD62L^{hi}/CD45RB^{hi}$) from memory/effector ($CD62^{lo}/CD45RB^{lo}$) T lymphocytes in a variety of murine systems (20–22). Interestingly, T cells recovered from the pleural cavity of IL-4-treated mice expressed significantly higher levels of both CD62L and CD45RB than did T cells from either saline- or IL-10-treated mice (Fig. 5A and 5B). Higher expression levels of these two differentiation markers were observed on both CD4 and CD8 T cell populations in IL-4-treated animals (unpublished data). These observations imply that the T cells recruited to the pleural cavity in IL-4-treated animals are impaired in their ability to mediate effector functions, such as γIFN production, resulting in a significant abrogation of the inflammatory response.

DISCUSSION

In the present report we have characterized an antigen-mediated *in vivo* model of leukocyte trafficking to the pleural cavity and correlated this response with local cytokine and chemokine expression. We observed that leukocyte recruitment to the pleural space is time- and cell-dependent (Fig. 1), with a pronounced 6 h response predominantly involving PMN migration and the upregulation of a number of chemokines, as well as IL-1β and TNFα expression. By 24 h, the number of macrophages and T cells present in the pleural cavity began to increase, concominant with an increase in γIFN levels in pleural exudate, while the expression of many chemokines and proinflammatory cytokines abated. At 48–72 h after antigen challenge, the number of PMNs was significantly reduced, the number of macrophages and T cells continued to increase, and the expression of some molecules, most notably CCL5, was maintained. These observations suggest a tightly coordinated response to antigen challenge

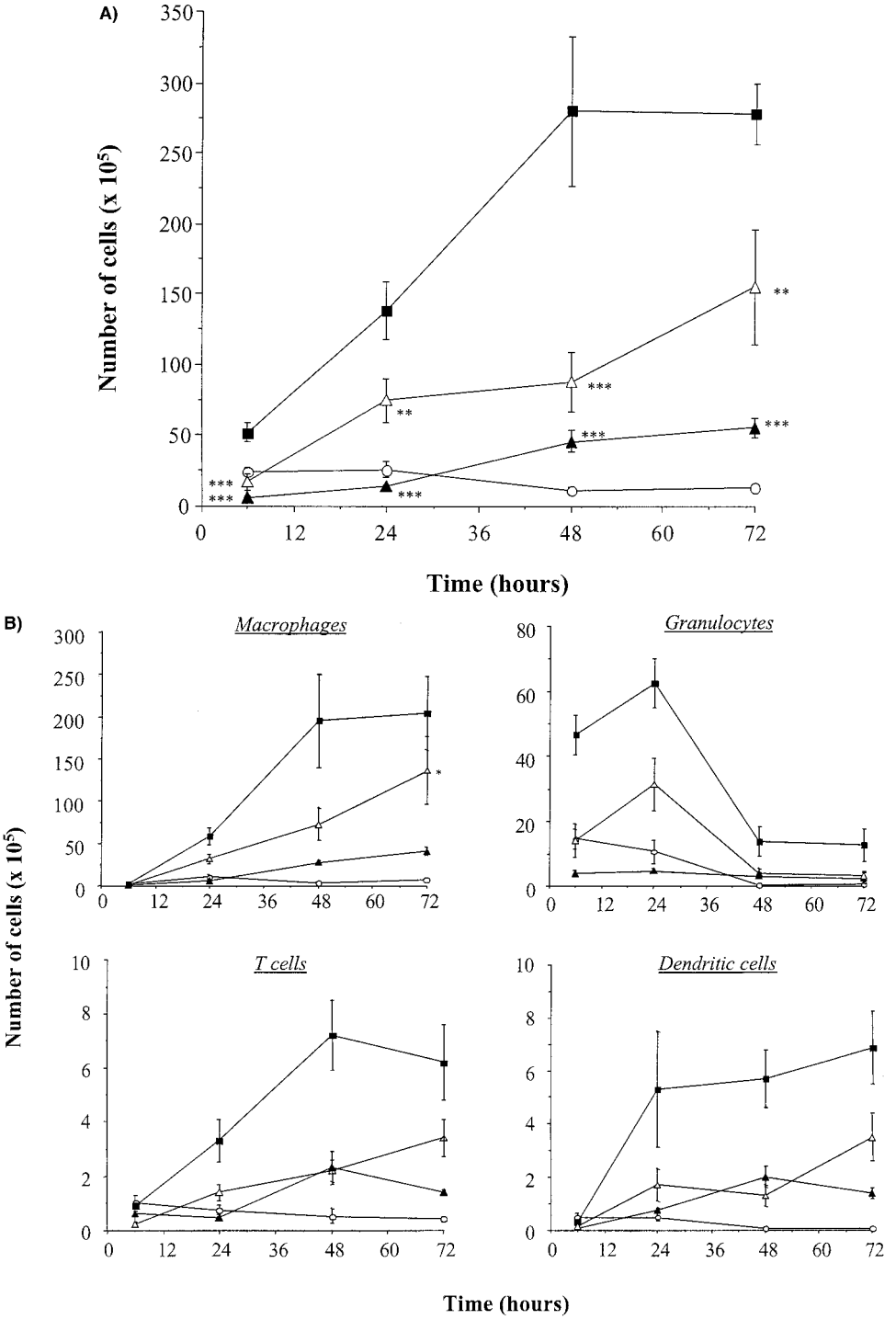

Fig. 4. Inhibition of leukocyte trafficking by IL-4 and IL-10 correlates with altered γIFN levels in the pleural cavity. A. Number of total leukocytes. B. Number of leukocyte subpopulations. C. Changes in γIFN levels. Open circle, no challenge; filled square, mBSA-challenge, saline-treated; open triangle, mBSA-challenge, IL-10-treated; filled triangle, mBSA-challenge, IL-4-treated. In A and C, statistically significant differences versus the saline-treated group are denoted as: $^{*}p < 0.05$; $^{**}p < 0.01$, $^{***}p < 0.001$. In B, all differences for each cell population from IL-4- and IL-10-treated groups compared to the saline-treated group at 24–72 h, and the difference for the granulocytes at 6 h relative to the saline group, are statistically significant at $p < 0.01$.

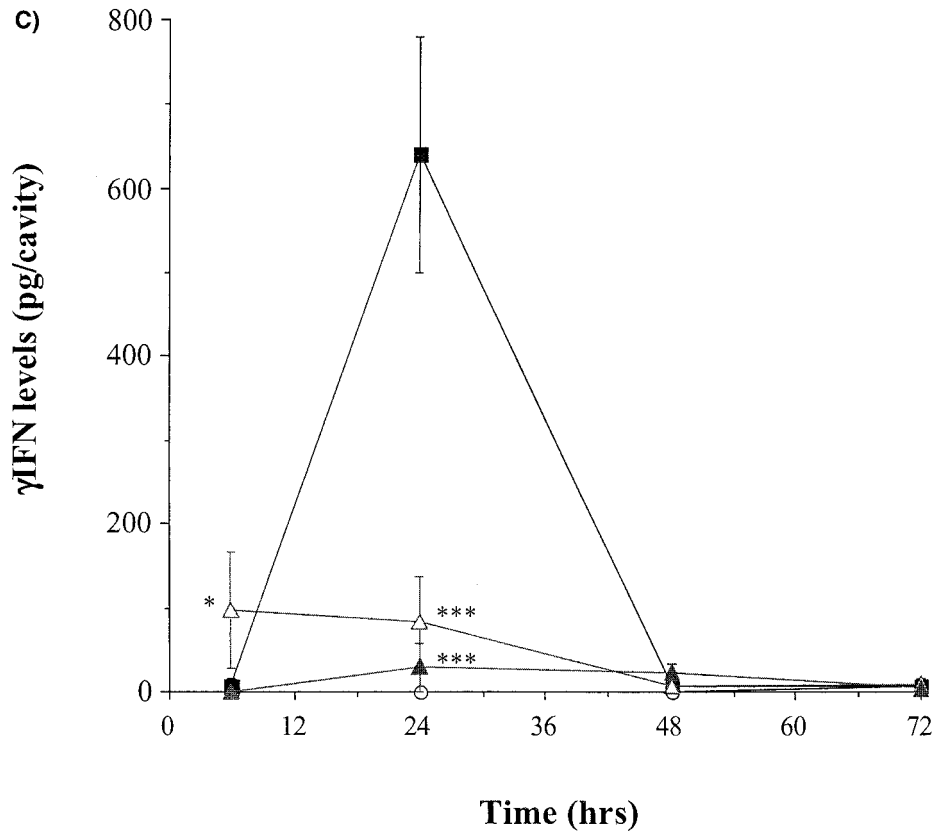

Fig. 4. (*Continued.*)

in sensitized animals at the levels of both leukocyte recruitment and proinflammatory cytokine and chemokine expression. Moreover, administration of IL-4 resulted in a marked inhibition of leukocyte migration to the pleural cavity and correlated with impaired γIFN production and the appearance in the pleural cavity of T cells with a naïve phenotype (Fig. 4). Treatment with standard anti-inflammatory compounds such as methotrexate, piroxicam, indomethacin, and dexamethasone are also effective in blocking leukocyte recruitment in this model (our unpublished observations). These results suggest that this model of leukocyte trafficking may be particularly useful in delineating the *in vivo* activity of novel inhibitors of leukocyte migration in antigen-induced inflammatory responses.

Our use of quantitative real-time PCR analysis enabled us to directly evaluate the kinetics of steady-state mRNA expression of numerous cytokines and chemokines (Fig. 2) and draw correlations with protein levels of these factors where appropriate. Many of the chemokines examined were expressed at exceptionally high levels at 6 h after challenge on a mRNA basis and then diminished with time. These included CXCL9, 10, and 11 (ligands for CXCR3), KC and MIP-2 (ligands for CXCR2), CCL2 (ligand for CCR2), and CCL3 and CCL4 (ligands for CCR1 and CCR5). We were able to confirm and extend our observations for KC and CCL2 mRNA expression to the examination of protein levels in pleural exudate (Fig. 3), suggesting that the early expression of these chemokines mediates the trafficking of the PMNs and monocyte/macrophages to the pleural cavity. The high initial expression of these chemokines may reflect their production by recently emigrated and activated PMNs within the pleural space (1, 2, 6). In contrast, chemokine expression for the most part appears to be inversely proportional to the numbers of monocyte/macrophages and T cells (Fig. 1B), suggesting that these leukocyte populations do not account for significant chemokine production in this model. A notable exception is CCL5 (a ligand for CCR1, 3, and 5), for which the mRNA and protein expression remained elevated through 72 h. This may reflect additional upregulation of CCL5 mRNA later in the T cell activation process (23) and/or

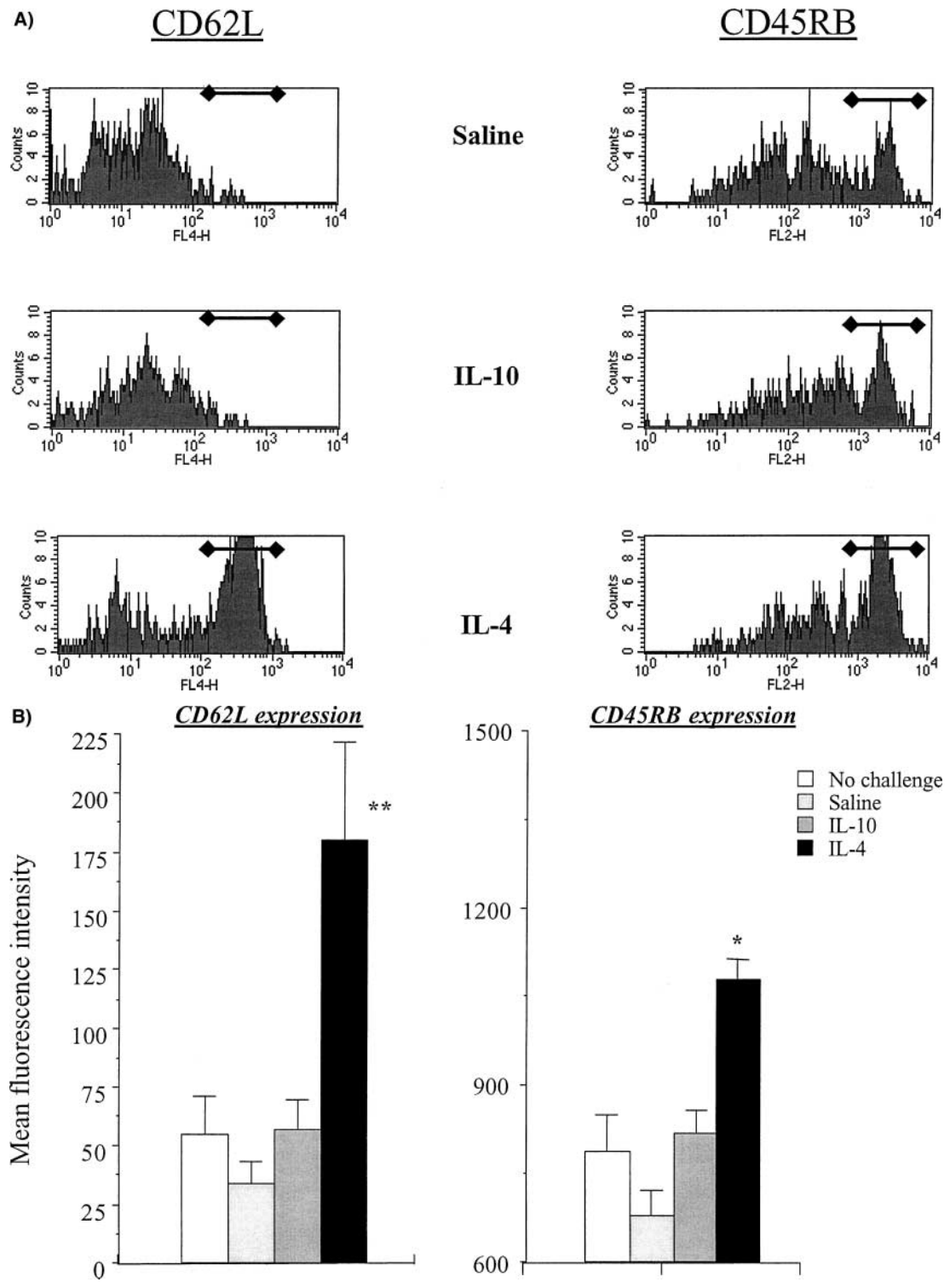

Fig. 5. Pleural cavity T cells express higher levels of CD62L and CD45RB following IL-4 administration. A. Left column, CD62L expression; right column, CD45RB expression. Representative data from mBSA-challenged mice treated with saline (top), IL-10 (middle), or IL-4 (bottom). Markers indicate highest expressing T cells. B. Mean ± SEM of treatment groups in A. T cells were evaluated 48 h after antigen challenge. $^{*}p < 0.05$ vs. saline treatment; $^{**}p < 0.01$ vs. saline treatment.

its production by different cell populations, possibly including nonleukocytes such as endothelium or fibroblasts. These observations suggest that CCL5 may play a critical role in mediating the accumulation of T cells and monocyte/macrophages in the pleural cavity at later timepoints (1, 2, 6). To date, we have been unable to directly test the importance of this chemokine in this response due to the lack of availability of an anti-murine CCL5 antibody with sufficient neutralizing activity.

The upregulation of cytokine mRNA levels was quantitatively less dramatic than was observed with chemokines (Fig. 2). We observed that IL-1β, IL-6, IL-12 p40, and TNFα mRNA levels were elevated at 6-h postchallenge and then diminished, relative to unchallenged controls, while γIFN mRNA and protein expression were maximal at 24 h. This peak of γIFN expression correlates with the initial increase in activated T cells trafficking to the cavity (Fig. 1B). The mild enhancement of the mRNA levels of IL-4, IL-10, IL-13, and TGFβ by pleural exudate leukocytes seen at later times was more sustained and may reflect the immunoregulatory activities of these cytokines (18, 19, 24). Given the substantial increase in the mRNA and/or protein levels of so many chemokines and cytokines in this system, it will be of interest to determine which molecules are essential for the induction of this response and which are redundant. These studies are presently underway.

Administration of exogenous IL-4 resulted in a significant inhibition of leukocyte trafficking to the pleural cavity, manifested as a complete blockade of PMN migration and dramatic reductions in the number of macrophages, T cells and dendritic cells (Fig. 4A and 4B). These results are in accord with previous reports in which IL-4 was shown to inhibit leukocyte migration in both air-pouch (25) and arthritis (26) models. We also observed a pronounced impairment of γIFN protein levels in the pleural exudate of IL-4-treated mice (Fig. 4C), consistent with the observation that IL-4-mediated inhibition of the development of a Th1-mediated DTH footpad response correlated with reduced γIFN production (27). Our results imply that this reduction in γIFN in the pleural cavity may be essential to the anti-inflammatory activity of IL-4 in this model. Treatment with IL-10 also inhibited leukocyte migration, but was less effective (Fig. 4A and 4B). Interestingly, while we found that IL-10 suppressed γIFN levels at 24 h after antigen challenge to a level similar to that seen following IL-4 treatment, we observed a mild elevation of γIFN levels in the pleural space at 6-h postchallenge in IL-10-treated mice (Fig. 4C). This immediate enhancement of γIFN by IL-10 may explain, at least in part, the difference in efficacy between IL-4 and IL-10 in this model. We were unable to detect significant and reproducible changes in chemokine expression following IL-4 or IL-10 treatment, suggesting that regulation of chemokine expression (28) may not contribute to this discrepancy.

Analysis of surface marker expression on T cells in the pleural cavity revealed a marked and selective increase in the expression of both CD62L and CD45RB in IL-4-treated mice, which was not seen in animals administered IL-10 (Fig. 5). Expression of these proteins has been shown to be elevated on naive T cells and to be downregulated on T cells with memory/effector function (20–22). These observations suggest that systemic IL-4 administration altered the ability of T cells in the draining lymph nodes to respond to the antigen challenge, resulting in the migration to the pleural cavity of T cells with reduced cytokine-producing and effector capabilities. Our data showing reduced pleural levels of γIFN following IL-4 treatment are consistent with this interpretation. That we were unable to identify elevated levels of Th2 cytokines in the pleural exudates from IL-4-treated animals implies that IL-4 treatment may not have deviated the immune response toward a Th2 response as much as it suppressed the generation of Th1 responses. These increased surface levels of CD62L and CD45RB do not appear to be due to a direct effect of IL-4, since this cytokine does not appear to modulate their expression (29, 30), although indirect effects of IL-4 on these molecules mediated by subtle changes in the pleural microenvironment cannot be excluded. It also remains formally possible that the regulation of the *in vivo* expression of CD62L and CD45RB is especially complex and undergoes significant changes that do not directly correlate with T cell function at the site of inflammation, as has been implied from *in vitro* studies for CD62L (31). Of note, IL-4 administration did not alter the expression of other markers that have been implicated in T cell activation, such as CD11a, CD25, or CD44 (unpublished observations). Additional evaluation of leukocyte surface markers, including chemokine receptors, and the expression of additional chemokines following both IL-4 and IL-10 therapy is currently in progress. It is tempting to speculate that the induction of decoy chemokine receptor expression by IL-10, and perhaps by IL-4 as well, may contribute to the inhibition of leukocyte trafficking in this model as well (32).

Many models of hapten- or antigen-mediated inflammation, such as those involving the skin (33–35), colon (35–37), or joint/paw (10–15, 27), typically require histopathological assessment, enzymatic assays (e.g.,

myeloperoxidase for PMNs) or lengthy cell isolation procedures to prepare and enumerate leukocyte accumulation at sites of inflammation. In the pleuritis model described here, leukocyte migration is rapidly and accurately quantitated, and alterations to individual cell populations readily identified using flow cytometric or cytological techniques. Moreover, the time-dependent regulation of cytokine and chemokine expression reported here is consistent with observations made in other inflammatory systems suggesting that this model may have utility for evaluating an array of novel therapeutics with anti-inflammatory activity.

Acknowledgments—We thank Ms Heather Byrnes for excellent technical assistance and Dr Satwant Narula for support and helpful scientific discussions.

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