IL-6 trans-signaling via STAT3 directs T cell infiltration in acute inflammation

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Edited by Philippa Marrack, National Jewish Medical and Research Center, Denver, CO, and approved May 23, 2005 (received for review March 4, 2005)

Interleukin (IL)-6 signaling through its soluble receptor (IL-6 trans-signaling) directs transition between innate and acquired immune responses by orchestrating the chemokine-directed attraction and apoptotic clearance of leukocytes. Through analysis of mononuclear cell infiltration in WT and IL-6-deficient mice during peritoneal inflammation, we now report that IL-6 selectively governs T cell infiltration by regulating chemokine secretion (CXCL10, CCL4, CCL5, CCL11, and CCL17) and chemokine receptor (CCR3, CCR4, CCR5, and CXCR3) expression on the CD3+ infiltrate. Although blockade of IL-6 trans-signaling prevents chemokine release, chemokine receptor expression remained unaltered suggesting that this response is regulated by IL-6 itself. To dissect the signaling events promoting T cell migration, inflammation was established in knock-in mice expressing mutated forms of the universal signaling molecule gp130. In mice (gp130Y757F/Y757F) deficient in SHP2 and SOCS3 binding, but presenting hyperactivation of STAT1/3, T cell recruitment and CCL5 expression was enhanced. Conversely, both of these parameters were suppressed in mice with ablated gp130-mediated STAT1/3 activation (gp130−/−). T cell migration was related to STAT3 activity, because monoallelic deletion of Stat3 in gp130−/−Y757F/Y757F mice (gp130−/−Y757F/Y757F; Stat3−/−) corrected the exaggerated responses observed in gp130−/−Y757F/Y757F mice. Consequently, STAT3 plays a defining role in IL-6-mediated T cell migration.

chemokines | cytokines | gp130

Interleukin (IL)-6 is traditionally considered a regulator of acute-phase responses and a lymphocyte stimulatory factor (1). However recent advances have highlighted a pivotal role for this cytokine in directing leukocyte trafficking and facilitating transition between innate and acquired immune responses (2, 3). Identification of these IL-6 activities has largely been achieved through an increased understanding of the regulatory properties of its soluble receptor, whereas better appreciation of IL-6 signaling has led researchers to consider the interplay between IL-6 and other cytokines (4–7). These studies collectively underline a role for IL-6 in governing inflammation and have emphasized the therapeutic potential of targeting IL-6 as a strategy for the management of infectious and inflammatory diseases (8, 9).

IL-6 responses are transmitted through gp130, which serves as the universal signal-transducing receptor subunit for all IL-6-related cytokines (10, 11). Although this classically occurs through IL-6 binding to its membrane-bound receptor (IL-6R), it is clear that a soluble form of the cognate IL-6 receptor (sIL-6R) affords IL-6 with an alternative mechanism of gp130 activation. This additional mode of cell activation is termed IL-6 trans-signaling and results from formation of a sIL-6R/IL-6 complex, which can directly bind cellular gp130 (2). Because gp130 is ubiquitously expressed within tissue, trans-signaling provides IL-6 with the capacity to activate cells that would not intrinsically respond to IL-6 itself (2).

Studies have documented inherent roles for IL-6 trans-signaling in a number of biological processes, including its involvement in leukocyte trafficking and activation (2, 3). Although initial observations in IL-6-deficient (IL-6−/−) mice noted that IL-6 suppressed neutrophil accumulation at sites of infection or inflammation (12, 13), it is evident that neutrophil clearance and their subsequent replacement by a more sustained population of mononuclear leukocytes is governed by the sIL-6R (5, 14). In particular, IL-6 trans-signaling defines the nature of the inflammatory infiltrate by controlling leukocyte apoptosis and the expression of inflammatory chemokines and adhesion molecules (5, 13–18). This event is a critical step in the successful resolution of any inflammatory response and defines an immunological switch from innate to acquired immunity at sites of inflammation.

In terms of mononuclear leukocytes, IL-6-mediated signaling is associated with the activation of a number of cellular events important in host defense and chronic disease progression. With respect to monocytes/macrophages, IL-6 affects cellular differentiation (19–21). However, the role of IL-6 in lymphocyte activation may have more profound consequences, where it controls T cell polarization (22–24), IL-2-dependent cell proliferation (25, 26), L-selectin (CD62L) adhesion (18), B cell activation, and antibody production (25). Furthermore, the ability of IL-6 to rescue T cells from entering apoptosis (17, 27–30) may have a considerable bearing in the progression of chronic inflammation. Indeed, sIL-6R-mediated trans-signaling promotes T cell expression of antiapoptotic regulators (17, 30), whereas blockade of sIL-6R signaling in experimental models suppresses the pathogenesis of Crohn’s disease and rheumatoid arthritis (17, 31). Through in vivo analysis of experimental peritoneal inflammation, studies presented herein now show that IL-6 activation of STAT3 promotes T cell recruitment.

Methods

Mouse Strains. Experiments were performed in weight-matched 7- to 12-week-old IL-6−/− mice (32), various knock-in gp130 mutant strains, and genetically matched wild-type (WT) controls. Engineering of homozygous gp130−/− mice and gp130Y757F/Y757F mice, and the compound gp130−/−Y757F/Y757F; Stat3−/− mice has been described in refs. 11, 19, 33, and 34. Procedures were performed in accordance with Home Office approved project license PPL-40/2131.

Staphylococcus epidermidis (SES)-Induced Peritoneal Inflammation. Peritoneal inflammation was induced in mice through i.p. administration of a cell-free supernatant prepared from a clinical isolate of S. epidermidis (SES) (5, 14). At defined intervals, the peritoneal cavity was lavaged, and the leukocyte infiltrate assessed by direct counting (Coulter Z2, Beckman Coulter), differential cell staining, and flow cytometric analysis. Lavage...
fluids were rendered cell-free by centrifugation for analysis of inflammatory mediators. Soluble gp130 was purchased from R & D Systems and was added as indicated.

Murine Leukocyte Isolation. Unfractionated hematopoietic cells from peripheral blood, spleens, and femoral bone marrow of mice were rendered free from red blood cell contamination by using an ammonium chloride lysis buffer (155 mM NH4Cl/7 mM K2CO3/0.1 mM EDTA) and washed in PBS before antibody labeling for flow cytometry.

Antibodies. Fluorochrome-conjugated antibodies were purchased from the following sources: rat anti-mouse monoclonal antibodies to CD3 (17A2), CD4 (GK1.5), CD8a (53–6.7), CD69 (H1.2F3), CD25 (PC61), CD28 (37.51), CD62L (MEL-14), CCR5 (C34–3448), CXCR5 (2G8), B220 (RA3–6B2), and IL-6R (D7715A7) were from BD Pharmingen. Phytoerythrin-conjugated rat anti-mouse monoclonal antibodies to CXCR3 (220803) and CCR3 (83101.111) were from R & D Systems. Murine polyclonal anti-CCR4 (sc-7936) (Santa Cruz Biotechnology). Murine polyclonal anti-CCR4 (sc-7936) (Santa Cruz Biotechnology).

Results

Flow Cytometric Analysis. Leukocytes were incubated with mouse Fc block (BD Pharmingen) before immunolabeling for 30 min at 4°C with primary fluorochrome-conjugated or nonconjugated antibodies. Where necessary, cells were incubated for a further 30 min at 4°C with appropriate fluorochrome-conjugated secondary antibodies. Cells were analyzed by using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) based on previously defined murine leukocyte settings. Data were acquired from 10,000 gated events and staining was compared with fluorochrome-conjugated isotype control antibodies.

ELISA Determinations. Murine CCL3 (MIP-1α), CCL5 (RANTES), CCL11 (eotaxin), CCL17 (TARC), CXCL10 (IP-10), and CXCL13 (BCA-1) were quantified by using commercially available ELISA kits (R & D Systems).

Western Blot Analysis of Peritoneal Lining. Protein lysates were prepared from frozen sections of parietal peritoneal membrane. Samples were cleared of cellular debris and separated by SDS-PAGE for Western blot analysis with specific primary antibodies (19). Immunolabeled proteins were detected by using appropriate HRP-conjugated secondary antibodies, followed by visualization with enhanced chemiluminescence (Amersham Pharmacia Biotech).

Statistical Analysis. Data are expressed as mean ± SEM, and statistical analysis were performed by using a Student’s unpaired t test (STATVIEW SE + GRAPHICS 1.03 software, Abacus Concepts, Berkeley, CA). P < 0.05 was considered significantly different.

Results

IL-6-Deficient Mice Exhibit Impaired T Cell Recruitment in Vivo. After induction of peritoneal inflammation in WT and IL-6−/− mice by using a cell-free supernatant (termed SES) derived from a clinical isolate of S. epidermidis, it was evident that IL-6 deficiency results in an impaired recruitment of lymphocytes but not monocytic cells (Fig. 1). This reduction in lymphocyte migration was confined to the CD3+ population, with no significant difference observed in the trafficking of B220+ B-cells (Fig. 1). Specific phenotypic analysis of the T cell population from WT and IL-6−/− mice showed that the composition of the CD3+ subpopulation was largely unaffected by the absence of IL-6, suggesting that the impaired infiltration was not attributable to a global T cell defect (see Tables 1 and 2, which are published as supporting information on the PNAS web site).
increase in CD3⁺ T cells expressing CCR3, CCR4, CCR5, and CXCR3, the proportion of cells bearing these receptors was noticeably lower in the absence of IL-6. Furthermore, the profile of expression for each chemokine receptor was distinct and does not parallel the recruitment of CD3⁺ cells presented in Fig. 1. This finding suggests that T cell chemokine receptors are differentially and temporally regulated during the course of an inflammatory response.

Dysregulation of chemokine-mediated T cell trafficking was substantiated by quantification of chemokine levels in peritoneal lavage fluid. These analyses showed that IL-6 deficiency is associated with defective expression of CCL4, CCL5, CCL17, and CXCL10, whereas a marginal difference was observed for CCL11 (Fig. 3A). Administration of WT mice with sgp130 (the natural antagonist for sIL-6R-mediated signaling) reverted the pattern of chemokine expression to that observed in IL-6⁺/⁻ mice (Fig. 3A), and dose-dependently suppressed lymphocyte migration into the peritoneal cavity (Fig. 3B). Although sgp130 blocked peritoneal chemokine expression, analysis of CCR5 and CCR3 expression on the CD3⁺ infiltrate showed that blockade of IL-6 trans-signaling did not affect the T cell expression of these chemokine receptors (Fig. 4). Similar data for CCR4 and CXCR3 were also obtained (data not shown). To test whether the defect in chemokine and cellular chemokine receptor expression was a universal feature of IL-6⁻/⁻ mice, CXCR5 levels on the B220⁺ population was monitored in conjunction with the peritoneal expression of its ligand CXCL13. As shown in Fig. 5, IL-6 deficiency did not affect the proportion of B220⁺ CXCR5⁺ cells or CXCL13 expression.

![Graph](https://via.placeholder.com/150)

**Fig. 2.** T cell chemokine receptor expression after peritoneal inflammation. SES-induced peritoneal inflammation was established in WT and IL-6⁻/⁻ mice. At defined intervals, the peritoneal cavity was lavaged and the CD3⁺ infiltrate analyzed by flow cytometry for changes in CCR3, CCR4, CCR5, and CXCR3. Values represent the mean ± SEM (n = 10 mice per group; *, P < 0.05).

![Graph](https://via.placeholder.com/150)

**Fig. 3.** Peritoneal chemokine levels after SES-induced inflammation. (A) SES-induced peritoneal inflammation was established in WT and IL-6⁻/⁻ mice, and at defined intervals, the peritoneal cavity was lavaged and chemokine levels quantified by ELISA. To block IL-6 trans-signaling, WT mice were administered with 150 ng per mouse sgp130. (B) Dose-dependent blockade of SES-induced lymphocyte recruitment (48 h) by sgp130 administration of WT mice. Values represent the mean ± SEM (n = 5 mice per group; *, P < 0.05).

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**Fig. 4.** Affect of sgp130 on T cell chemokine receptor expression. SES-induced peritoneal inflammation was established in IL-6⁻/⁻, WT, and WT mice treated with sgp130. After 36 h, the peritoneal cavity was lavaged and the proportion of CD3⁺ CCR3⁺ and CD3⁺ CCR5⁺ cells determined. Representative scatter plots for each condition are also shown. Values represent the mean ± SEM (n = 5 mice per group; *, P < 0.05).
Genetic modification of gp130-mediated STAT3 signals affects T cell recruitment after SES-induced inflammation. SES-induced peritoneal inflammation was established in Stat3 heterozygous (gp130Y757F/Y757F; Stat3+/-) background (11). To verify the specific reduction in peritoneal STAT3 expression within gp130Y757F/Y757F; Stat3+/- mice, STAT3 levels and its activation after SES stimulation was monitored by Western blot analysis in peritoneal membranes from the various mouse strains tested (Fig. 6C). Consistent with previous results (34), cellular STAT1 levels and its initial activation remained unaffected by the monoallelic deletion of STAT3 (Fig. 6C). Quantification of T cell infiltration in gp130Y757F/Y757F; Stat3+/- mice showed that reduction of gp130-mediated STAT3 signaling reduced the exaggerated T cell migration seen in gp130Y757F/Y757F; Stat3+ mice (Fig. 6A) and suppressed CCL5 expression back to that observed in WT mice (Fig. 6B). The specificity of these STAT3-mediated responses is further suggested by experiments that demonstrate that gp130 activation of peritoneal mesothelial cells selectively activates STAT3 but not STAT1 (35). Thus, the defect in T cell trafficking seen in IL-6-deficiency appears to require gp130-mediated STAT3 activation.

**Discussion**

Transition from innate to acquired immunity represents a pivotal event in the control of any inflammatory response. Regulation of this immunological switch relies on the coordinated expression of chemotactic agents and a precise regulation of apoptotic events. Through differential control of these processes, recent studies have highlighted that gp130 signaling has the capacity to suppress innate immune responses (12, 14, 23) while concurrently promoting development of acquired immunity (18, 23, 25–27). In this study, we have extended these observations by demonstrating that IL-6 deficiency specifically disrupts chemokine control of T cell trafficking, yet leaves monocytic and B cell recruitment unaffected. This response is somewhat surprising given that many of the inflammatory chemokines shown to be dysregulated in IL-6−/− mice also attract mononuclear cells. Because IL-6 also modulates the balance between macrophage and dendritic cell differentiation (19–21), it remains to be established whether IL-6 deficiency has specific affects on the phenotype of a recruited mononuclear cell population. Consequently, the nature of the monocytic cells may be as important as the total cell numbers. Dysregulated T cell migration is however consistent with the role IL-6 performs in more chronic inflammatory conditions, where the ability of IL-6 to rescue T cells from
entering apoptosis has been linked with the pathology of several models of chronic disease (4, 17, 36).

Although IL-6 has been defined as a T cell activation factor and shown to induce T cell proliferation and survival (27–30), it is now evident that IL-6 bioactivity is also tightly linked to the inflammatory response and is critical to the maintenance of immunity. IL-6 is produced mainly by activated T and B cells, as well as monocytes/macrophages, and can induce T cell proliferation and survival. IL-6 has also been shown to stimulate the production of pro-inflammatory cytokines such as TNF-α and IL-1β, which are known to promote T cell activation (21, 22).

The expression of IL-6 is tightly regulated by the intracellular signaling pathways triggered by T cell activation. For instance, lymph nodes cells from IL-6−/− mice have reduced expression of IL-2 (22), which is known to promote T cell activation. IL-6 has also been shown to induce the expression of several chemokines, including MIP-1α and MIP-1β, which are known to attract T cells to sites of inflammation. Furthermore, IL-6 has been shown to induce the expression of several chemokine receptors, including CCR5 and CXCR4, which are known to be involved in T cell migration to sites of inflammation.

In conclusion, IL-6 plays a critical role in the regulation of T cell activation and survival. Its expression is tightly regulated by the intracellular signaling pathways triggered by T cell activation, and its bioactivity is tightly linked to the inflammatory response. IL-6 has been shown to promote T cell activation, induce the expression of several chemokines, and regulate the expression of several chemokine receptors, all of which are known to play a critical role in T cell migration to sites of inflammation. The therapeutic targeting of IL-6 may have significant implications for the treatment of chronic inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, which are associated with the protection of the central nervous system from inflammation.

This work was supported through The Wellcome Trust and Arthritis Research Campaign grant projects.


