Stat4 Is Expressed in Activated Peripheral Blood Monocytes, Dendritic Cells, and Macrophages at Sites of Th1-Mediated Inflammation

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Stat4 Is Expressed in Activated Peripheral Blood Monocytes, Dendritic Cells, and Macrophages at Sites of Th1-Mediated Inflammation

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Stat4 is a key transcription factor involved in promoting cell-mediated immunity, whose expression in mature cells has been reported to be restricted to T and NK cells. We demonstrate here, however, that Stat4 expression is not restricted to lymphoid cells. In their basal state, monocytes do not express Stat4. Upon activation, however, IFN-γ and LPS-treated monocytes and dendritic cells express high levels of Stat4. Monocyte-expressed Stat4 in humans is phosphorylated in response to IFN-α, but not IL-12. In contrast, the Th2 cytokines, IL-4 and IL-10, specifically down-regulate Stat4 expression in activated monocytes, while having little effect on Stat6 expression. Moreover, macrophages in synovial tissue obtained from patients with rheumatoid arthritis express Stat4 in vivo, suggesting a potential role in a prototypical Th1-mediated human disease. IFN-α-induced Stat4 activation in human monocytes represents a previously unrecognized signaling pathway at sites of Th1 inflammation. The Journal of Immunology, 2000, 164: 4659–4664.

Cell-mediated immunity is critical for host defense against intracellular infections and the pathogenesis of certain autoimmune diseases. This response is dependent upon the production of IL-12 by macrophages, neutrophils and dendritic cells (DC),3 which in turn stimulates NK cell activity and the production of IFN-γ. IL-12 and IFN-γ then act to drive expansion of a subset of CD4 T cells (Th1 cells) characterized by production of IFN-γ and lymphotoxin (1–3). Stat4 is a key molecule in the intracellular signal transduction cascade activated in response to IL-12 stimulation. It is phosphorylated in response to IL-12, resulting in the translocation of Stat4 to the nucleus and transactivation of genes that presumably promote Th1 differentiation (4, 5). Stat4-deficient mice highlight the central role of Stat4 in mediating IL-12 signals, as they have major defects in IFN-γ production, NK cell activity, IL-12-mediated proliferation, and Th1 differentiation (6, 7).

Yet, in humans, Stat4 is not only activated by IL-12. In human lymphoid cells, unlike in murine cells, it is also phosphorylated by IFN-α, demonstrating that the use of Stat4-dependent Th1 differentiation pathways in humans may not be under the exclusive control of IL-12 (8, 9). IFN-α also acts to enhance the Th1 inflammatory response by increasing NK activity and promoting Th1 differentiation of T cells (9, 10). Although the exact role of Stat4 in the positive regulation of Th1 inflammation by IFN-α has not been defined, the possibility exists that, in addition to IL-12, IFN-α modulates cellular immunity via Stat4.

Stat4 was initially reported to be expressed in the thymus, spleen, and testes. However, its role in testicular development is evidently inconsequential; only T cell and NK cell abnormalities were reported in Stat4 knockout mice. Immature myeloid cell lines were also shown to express Stat4, but it was reported that differentiated myeloid cells lacked Stat4 (11). Thus, all subsequent studies focused on T cells and NK cells. However, peripheral blood monocytes, macrophages, and DC play pivotal roles in cell-mediated immunity. We therefore asked whether Stat4, a key transcription factor that promotes cellular immunity, was expressed and functional in these cells of monocyte lineage. Stat4 expression in T cells is greatly influenced by their state of activation. Human peripheral blood T cells do not have Stat4 in their basal state, but its expression is markedly induced following stimulation (4). Based on this result, we hypothesized that other immune cells, including differentiated cells of monocyte lineage, may only express Stat4 upon activation.

We demonstrate in this paper for the first time that Stat4 is present in activated monocytes and DC. Monocyte-expressed Stat4 is activated in response to IFN-α, but not to IL-12. We further show that Stat4 is abundantly expressed in synovial macrophages in patients with rheumatoid arthritis (RA), an autoimmune disease characterized by Th1 inflammation.

**Materials and Methods**

**Cell purification and culture**

Monocytes were purified from the peripheral blood of normal volunteers by elutriation (Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD). In selected experiments, these monocytes were further purified by depleting the nonmonocytic population.
with commercially available immunomagnet beads using the manufacturer’s protocol (Miltenyi Biotec, Auburn, CA). Isolated monocytes were stained with fluorochrome-conjugated CD3, CD14, CD16, CD19, and CD56 Abs, or the respective conjugate-matched, isotype-matched irrelevant control Abs (Becton Dickinson, San Jose, CA), followingFc receptor blockade with FCS and human Ig. The resulting cells were consistently >99% CD14+ monocytes with <1% contamination with B cell, T cells, or NK cells by flow cytometry.

Purified monocytes were cultured in complete RPMI (c-RPMI; RPMI supplemented with 10% heat-inactivated FCS (Biofluids, Rockville, MD)), 2 mM L-glutamine (Biofluids) and 100 U/ml Pen-Strep (Biofluids) in 5 ml cultures in 6 well polystyrene tissue culture plates (Becton Dickinson). In some cases, monocytes were activated with 1000 U/ml INF-γ (Biological Resource Bank, National Cancer Institute, Frederick, MD), 200 ng/ml LPS (Sigma, St. Louis, MO), and/or 1000 U/ml INF-α (Biological Resource Bank, National Cancer Institute). As indicated, cytokine neutralizations were performed using goat polyclonal Abs (R&D Systems, Minneapolis-MN) as follows: 20 μg/ml anti-IL-4, 20 μg/ml anti-IL-10, and 10 μg/ml anti-IL-12. Additionally, conditioned medium was obtained from monocytes stimulated with LPS and INF-γ for 36 h. This conditioned medium was added to cultures in the indicated experiments to examine the role of soluble factors in Stat4 induction.

DC were generated by culturing purified monocytes (2 × 10⁶ cells/ml) for 7 days in c-RPMI supplemented with IL-4 and GM-CSF (1000 U/ml and 5 ng/ml, respectively; R&D Systems). A portion of these cells was evaluated by flow cytometry for purity and found to be >99% CD14+ (Becton Dickinson) and predominantly CD1a+ (PharMingen, San Diego, CA). The cells were then cultured for an additional 24 h, with selected wells stimulated with INF-γ and LPS, as described above.

**RNase protection assays**

RNA from unstimulated and LPS and/or INF-γ-stimulated monocytes (10⁵) was prepared using the RNA STAT-60 kit (Tel-Test, Friendswood, TX). The GAPDH and L32 housekeeping control probes were obtained from a commercial source (PharMingen). Twenty percent of the RNA obtained from the preparations described previously was used for each RNase protection assay, performed using the PharMingen protocol. The Stat4 probe used in the indicated blot was generated by cloning a Stat4 RT-PCR product into the PCR 2.1-TOPO TA cloning vector (Invitrogen, Carlsbad, CA). Reverse transcription was performed using a commercial kit (Boehringer Mannheim, Indianapolis, IN). The PCR was conducted with a 60°C annealing temperature and primers were used as follows: 5′-GAGGCAAGCT TCTAACAATGGAAC-3′ and 5′-CTCTGAATCCGTTCTGAA-3′.

**Immunoprecipitation and Western blot analysis**

Following incubation, the monocyte culture plates were placed on ice for 25 min. The monocytes were removed from the plate by gentle scraping, sedimented, and lysed in buffer containing Triton X-100. Stat4 immunoprecipitation and Western blotting using polyclonal rabbit anti-huStat4 (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA), anti-huStat6 (1:1000, Santa Cruz Biotechnology), or anti-phosphorytosine (4G10, 1:1000; Upstate Biotechnology, Lake Placid, NY) were performed as described (12).

**Immunofluorescent staining**

Elutriated monocytes adhered to coverslips inserted in the wells of a 24-well plate at a density of 5 × 10⁵ cells/ml and were stimulated with 200 ng/ml INF-γ and 100 U/ml INF-α for 24 h. The coverslips were washed twice with PBS, fixed for 2 min with 50%/50% ice-cold methanol/acetic acid, washed three times with PBS, and blocked with PBS supplemented with 1% BSA (Sigma), 5% goat serum (Life Technologies, Grand Island, NY), and 1 mg/ml gamma globulin (Sigma). Primary and secondary staining was conducted at room temperature for 1 h in PBS supplemented with 1% BSA, 5% goat serum, and 20 μg/ml human gamma globulin. The following Abs were used for primary staining at 1 μg/ml: polyclonal Stat4 Ab (as above) and monoclonal anti-CD14 (mAb 63d3, provided by Dr. Steven Shaw, National Cancer Institute). As a negative control for the polyclonal Ab, purified rabbit IgG (Jackson ImmunoResearch, West Grove, PA) at 5 μg/ml, while LPS (mixture of anti-CD14, IgG1, and IgM, provided by Dr. Steven Shaw) was used as monoclonal control at 1 μg/ml. This was followed by a total of five washes with PBS supplemented with 1% BSA. Affinity purified goat anti-rabbit IgG-FITC and goat anti-mouse-IgG-Cy5 (Jackson ImmunoResearch) were used at 5 μg/ml as secondary Abs. The coverslips were washed as before and air-dried. After the coverslips were mounted on slides using “Permount” (Molecular Probes, Eugene OR), staining was analyzed on a Zeiss LSM 410 laser scanning confocal microscope with a ×63, planapochromat objective (N.A. 1.4), using 488 nm krypton/argon and 633 nm helium/neon laser excitation. Fluorescence was collected using two photomultiplier tubes using 510–540 nm and 670–810 nm band pass emission filters for FITC and CY5, respectively. Positive samples and negative controls were viewed using the same contrast and brightness settings.

**Immunohistochemical staining of tissue samples**

Synovial tissue was obtained at joint replacement surgery from three patients who met 1987 American College of Rheumatology criteria for RA. Appropriate patient consent was obtained. Frozen tissue sections were cut in 8-micron sections, affixed to slides, and fixed in chilled acetone for 15 min. Immunohistochemical methods were employed utilizing the following Abs: polyclonal rabbit anti-huStat4 (0.4 μg/ml), monoclonal mouse anti-huCD14 (2.5 μg/ml), and rabbit IgG (0.4 μg/ml). Tissue sections were mounted in Tris-buffered saline (TBS), then blocked for 10 min with 0.35% hydrogen peroxide blocking solution. Following a brief wash, a blocking solution (25% normal goat serum and 25% pooled human serum in TBS) was applied, and slides were incubated in a humidified slide chamber for 30 min at room temperature. The blocking solution was removed and 100 μl of the diluted primary mAb was applied. Slides were then incubated overnight at 4°C in the slide chamber. Next, slides were washed twice in TBS, and 100 μl of the appropriate HRP-conjugated secondary Ab was applied to each slide for an incubation period of 60 min at room temperature. Following a final wash, the color substrate (Liquid DAB; Dako, Carpinteria, CA) was applied with color development allowed up to 10 min. The slides were washed in distilled water, counterstained with hematoxylin solution, and evaluated by light microscopy. Polyclonal rabbit anti-huStat4 Ab at 0.4 μg/ml was incubated with Stat4 control peptide (Santa Cruz Biotechnology) at a ratio of 1:10 (Ab:Ag) for 1 h at 37°C before application to each tissue section in neutralization studies. In double-staining experiments, tissue preparation and staining with rabbit anti-huStat4 Ab (0.6 μg/ml) was performed as described above, and color was developed using diaminobenzidine substrate. Slides were then washed twice, and mouse anti-huCD14 Ab was applied (2.5 μg/ml) for 2 h at room temperature in a humidified slide chamber. After two washes, a biotinylated goat anti-mouse Ab (Dako; used at 2.8 μg/ml) was applied for 1 h. Streptavidin alkaline phosphatase (Dako; used at 4 μg/ml) was applied to each slide again after two washes for a 1-h incubation. After a final wash, another substrate (New Fuchsin; Dako) was applied for color development. Slides were again washed in distilled water and evaluated as before.

**Results**

**Activated peripheral blood monocytes express Stat4 mRNA and protein**

In initial experiments to examine Stat4 expression in cells of monocyte lineage, we analyzed highly purified human monocytes (<1% T cell, NK, or B cell contamination), first measuring Stat4 mRNA by RNase protection assay. As shown in Fig. 1A, Stat4 message was negligible in unstimulated monocytes (lanes 1 and 2). INF-γ is a well-characterized activator of monocytes and a promoter of cell-mediated immunity (13). We therefore treated monocytes with INF-γ to determine whether Stat4 could be induced. Interestingly, stimulation of purified monocytes with INF-γ alone induced low level Stat4 expression (lane 3). LPS, another activator of macrophage function, also stimulated low level induction of Stat4 message (lane 4). Much higher levels of Stat4 expression were detected in monocytes cultured with both INF-γ and LPS (lane 5), an effect seen with many other monocyte genes. Indeed, the level of Stat4 in activated monocytes was equivalent to that of PHA-stimulated PBMC (lane 6), which are predominantly lymphocytes and known to express high levels of Stat4 (4).

To confirm that the Stat4 message that we observed was not due to expression in contaminating cells, we analyzed the monocyte preparations by immunofluorescence microscopy (Fig. 1B). Elutriated monocytes activated with INF-γ and LPS (CD14+ red, middle left and lower left panels) showed Stat4 staining (green, upper left and lower left panels). Specificity was confirmed by the absence of staining with control IgG under the same conditions (right panels). Finally, Stat4 production was demonstrated at the protein level by Western blotting in elutriated monocytes in a time course experiment (Fig. 1C). Although no Stat4 was detected at
rest (lane 2), the protein was detected following 24 h of stimulation with IFN-γ and LPS (lane 6).

Furthermore, the synergy between IFN-γ and LPS was observed at the protein level as well (Fig. 2). Following 2 days of culture, only very low level induction was seen in IFN-γ-stimulated (lane 3) or LPS-stimulated (lane 4) cultures of purified monocytes compared with unstimulated controls (lane 2). However, very high levels of Stat4 were present in monocytes stimulated with both IFN-γ and LPS (lane 5). For comparison, lysate from an NK cell line that expresses abundant levels of Stat4 is shown in lane 1. Taken together, our data provide strong and unambiguous evidence that activated monocytes express Stat4 at levels comparable to activated lymphocytes; the potent monocyte activators LPS and IFN-γ synergistically induce the expression of this transcription factor.

We next addressed whether Stat4 was directly induced by IFN-γ and LPS, or whether secondary factors secreted by monocytes were involved in this process. The induction of Stat4 protein production...
FIGURE 2. Stat4 regulation and phosphorylation in cultured human monocytes and DC. A. Immunoblot analysis of the regulation of Stat4 (upper panel) and Stat6 (lower panel) in activated monocytes and DC. In lanes 2–11, purified monocytes were incubated for 48 h under the indicated stimulation conditions. Equal amounts of protein (100 µg) were analyzed by immunoblotting. Lanes 13 and 14 show Stat4 in DC differentiated in vitro and stimulated as indicated for an additional 24 h with LPS and IFN-γ. NK3.3 lysates were assayed as a positive control for Stat4 expression (lanes 1 and 12). B, Stat4 phosphorylation in human monocytes. Purified monocytes were preincubated for 24 h with LPS (lanes 1–6) or LPS + IFN-γ (lanes 7 and 8) and then stimulated for varying amounts of time with IFN-α, as indicated. Stat4 was immunoprecipitated and evaluated for activation by staining with anti-phosphotyrosine (top panel). A Stat4 reblot was performed to confirm equal loading for each of the two prestimulation conditions (bottom panel).

was indirect, at least partially, as 36-h conditioned medium from LPS- and IFN-γ-treated monocytes accelerated the Stat4 induction kinetics in monocytes (Fig. 1D). Monocytes stimulated in the presence of conditioned medium (right lanes) showed at least an 8 h acceleration in Stat4 production kinetics compared with those cultured with LPS and IFN-γ alone (left lanes). Because levels of LPS and IFN-γ were present at saturating levels, this acceleration could not be accounted for by small differences in exogenous LPS and IFN-γ present in the cultures, implying a role for soluble, monocyte-derived factors in the up-regulation of Stat4 protein. One such factor produced by monocytes in response to combined LPS and IFN-γ stimulation is IL-12 (104 pg/ml by ELISA; data not shown). Blockade of IL-12 did result in a small reduction in the induction of Stat4 by LPS and IFN-γ (Fig. 1E, lane 6 vs lane 4), while control Ig had no effect (lane 5), suggesting the fascinating possibility of autocrine regulation of Stat4.

Th2 cytokines specifically down-regulate Stat4 expression in activated monocytes

In contrast to IFN-γ and LPS, cytokines such as IL-4 and IL-10 promote allergic responses and inhibit cell-mediated immunity (1). We therefore examined whether incubation of monocytes with either of these cytokines could regulate Stat4 expression. As shown in Fig. 2A, both IL-4 (lane 6) and IL-10 (lane 9) blocked the induction of Stat4 production by IFN-γ and LPS (lane 5). The Th2-derived cytokine effect on Stat4 induction was specific, as neutralizing Abs to IL-4 (lane 7) and IL-10 (lane 10), but not control IgG (lanes 8 and 11), reversed the blockade on Stat4 induction. For comparison, we examined regulation of Stat6 production in the same culture conditions (Fig. 2A, lower panel). The production of Stat6, a molecule critical for the Th2 immune response, was also inducible and up-regulated synergistically by IFN-γ and LPS (lane 5). However, Stat6 expression was unaffected by IL-4, and only modestly decreased by IL-10 (lanes 6 and 9), indicating that the blockade of Stat4 expression by these Th2-derived cytokines is relatively specific.

DC express Stat4 protein following activation

DC are an important component of Th1 responses and are major producers of IL-12 (14). IL-4, used in combination with GM-CSF, is a standard means to differentiate monocytes into immature DC (15). Based on the finding that treatment of monocytes with IL-4 inhibited Stat4 expression, we postulated IL-4-differentiated DC might also lack Stat4. Peripheral blood monocytes were cultured with IL-4 and GM-CSF for 7 days, at which time they developed the phenotype of DC by flow cytometry (CD14+, CD1a+). Importantly, DC produced by this standard protocol did not express Stat4 (Fig. 2A, lane 13). We therefore asked if DC could still be activated to express Stat4. Consequently, we activated DC with the same stimuli that induced Stat4 in monocytes, namely IFN-γ and LPS. DC activated by treatment with IFN-γ and LPS, like monocytes, were induced to express Stat4 (Fig. 2A, lane 14). Shown as a positive control for Stat4 expression is lysate from the NK 3.3 cell line (lane 12).

Stat4 in monocytes is phosphorylated in response to IFN-α

Stat-mediated transcriptional activation is dependent upon phosphorylation of a conserved C-terminal tyrosine residue. Dimerization then occurs through reciprocal phosphotyrosine/Src homology 2 (SH2) interaction, a requirement for subsequent DNA binding and gene induction (16, 17). Having shown that Stat4 was expressed in activated monocytes, we next investigated what conditions would result in its activation. Because IL-12 is the classical cytokine activating Stat4, we first sought to determine whether this cytokine could activate monocyte-expressed Stat4. As activated monocytes produce IL-12, this would provide an autocrine means of promoting cell-mediated immune responses. Nevertheless, although activated monocytes expressed detectable IL-12R β1 protein, no IL-12R β2 protein was detected by flow cytometry under any condition tested. Not surprisingly, therefore, we found no detectable IL-12-induced Stat4 phosphorylation in monocytes under these conditions (data not shown). However, because blockade of IL-12 reduced monocyte production of Stat4 (Fig. 1E), there exists the possibility that there may be a short-lived period of IL-12R β2 production during monocyte activation which allows this signaling to occur. Alternatively, high-affinity IL-12R could be present below the level of detection of FACS, but still at sufficient levels to allow for some signaling to occur.

Although IL-12 is a major activator of Stat4, it is not the only factor to induce Stat4 phosphorylation in humans; Stat4 has been shown to be phosphorylated in response to IFN-α in human lymphoid cells (8, 9). We therefore investigated whether IFN-α stimulation initiates phosphorylation of Stat4 in activated monocytes. Purified monocytes were pretreated with LPS (Fig. 2B, lanes 1–6) for 24 h to induce Stat4 production before stimulation of selected...
cultures with IFN-α for various periods of time. No phosphorylation of Stat4 was detected in unstimulated monocytes (lane 2), but monocytes stimulated with IFN-α showed phosphorylation of Stat4 at 30 min that declined over 4 h (lanes 3–5). Similarly, in cultures pretreated with LPS and IFN-γ to induce even higher levels of Stat4 production (lanes 7 and 8), phosphorylated Stat4 was detected following 2 h of IFN-α stimulations as well, although not increased when compared with LPS pretreatment alone. Within each group representing the two prestimulation conditions, equivalent levels of Stat4 measured by Western blot were immunoprecipitated from unstimulated cultures or those stimulated with IFN-α (Fig. 2B, bottom). The slower migrating band in the Stat4 reblot corresponded to phosphorylated Stat4.

**Stat4 is expressed by macrophages in rheumatoid synovium**

Although Stat4 was present and functional in activated monocytes in vitro, we wanted to determine whether this was relevant in vivo. That is, the question still remained whether monocyte-derived cells express Stat4 in vivo at sites of inflammation in human subjects. To address this question, we obtained tissue samples from actively inflamed joints of RA patients, a disease process marked by Th1-dominated inflammation. In sections of synovial membrane from affected RA patients, Stat4 staining was observed in the synovial lining cells (brown, Fig. 3A). Parallel sections stained for CD14 showed that the cells in this area were predominantly type A synoviocytes (macrophages) characteristic of rheumatoid synovial membrane (pink, Fig. 3B) (18). This finding was confirmed in parallel sections double-stained for both Stat4 and CD14, in which the cells clearly showed staining for both molecules (Fig. 3C). No Stat4 staining was observed in experiments including preincubation of the anti-Stat4 Ab with its cognate peptide (Fig. 3D), indicating that the observed Stat4 staining was specific. These data demonstrate that Stat4 expression occurs at sites of inflammation in humans, and is not simply an in vitro phenomenon.

**Discussion**

Stat4 is a key transcription factor with important functions in the generation of a cell-mediated immune response. While its expression in T cells and NK cells has been well studied, other roles have not been considered. We show here for the first time that Stat4 is expressed in activated cells of monocyte lineage, including both macrophages and DC. The significance of this finding is highlighted by its high-level expression in activated macrophages present at the site of inflammation in RA, a prototypical Th1 autoimmune disease and by its specific down-regulation by the Th2-derived cytokines, IL-4 and IL-10. We further demonstrate that Stat4 from LPS-stimulated monocytes cultured under our stimulation conditions is activated by IFN-α, but not by IL-12.

Taken together, our data indicate that the Stat4 production and phosphorylation are highly regulated in both myeloid and lymphoid cells. The Stat4 signal transduction pathway is not functional in unactivated cells of monocyte lineage, because Stat4 is not expressed. Only after activation is Stat4 present and able to transduce signals in cells of monocyte lineage, limiting the availability of this signal transduction pathway to sites of inflammation. Furthermore, because Stat4 production is negatively regulated by Th2 cytokines, Stat4-dependent IFN-α signaling may be restricted to inflammation characterized by Th1 predominance. Macrophages are known to develop different phenotypes and costimulatory functions depending on the balance between Th1 and Th2 cytokines present during culture (19). Our data demonstrate that the ability to transduce Stat4 signals likewise distinguishes monocytes grown in Th1 vs Th2 conditions. Moreover, our results may now explain why Stat4 has been reported not to be present in unactivated DC in other systems (14).

Interestingly, murine bone marrow-derived macrophages and DC have been reported to produce IFN-γ in response to IL-12 in the same manner as lymphoid cells (20, 21). Indeed, Stat4 is phosphorylated in response to IL-12 in murine macrophages and DC. Moreover, IL-12-dependent IFN-γ production in these cells is abrogated in Stat4-deficient mice (unpublished results). In contrast to murine DC, however, we did not observe IL-12R β2 protein in human monocytes, making Stat4-dependent IL-12 signaling unlikely. Nevertheless, other conditions may exist under which the IL-12 receptor components and Stat4 are coexpressed in cells of monocyte lineage. In addition, we have detected only very small
levels of IFN-γ production by purified peripheral blood monocytes and macrophages. Moreover, we did not detect IFN-γ production by human bone marrow-derived macrophages stimulated with IL-12 and IL-18, which is in stark contrast to levels produced by murine bone marrow-derived macrophages (unpublished results).

Naturally, the following question arises: Why do monocytes make Stat4 upon activation? The fact that IFN-α induces phosphorylation of Stat4 may address this question. IFN-α clearly has been demonstrated to promote cell-mediated immunity and can drive Th1 differentiation even in the absence of IL-12 (9, 10). It is possible that some of these functions require Stat4. Unfortunately, murine knockout models will have some limitations in assessing the function of monocyte-derived Stat4 in humans, as Stat4 is not activated in response to IFN-α in the mouse (9). Given the interspecies differences in IFN-α signaling, this issue will need to be addressed in other ways. Samples from human patients with Stat4 mutations, if they exist, will be useful for this purpose.

We speculate that Stat4 expression reflects a point of human monocyte differentiation, facilitating a new signal transduction pathway in the response to IFN-α. In the absence of IL-12-dependent Stat4 phosphorylation, IFN-α signaling via Stat4 may occur as part of the inflammatory process in RA. Indeed it appears that a majority of the macrophages present in the synovial lining layer of joints affected by RA express Stat4. Interestingly, a RA disease is reported in patients receiving IFN-α as therapy for malignancies (22). Because monocytes and their descendents are central to the granulomatous response to intracellular infections and in certain autoimmune diseases, our data suggest the intriguing possibility that Stat4 signaling in monocytes may be relevant in other disease processes. Stat4 gene targets could be predicted to overlap those involved in Th1 function of lymphocytes, but may also include genes specific only to myeloid cells. Therefore, determining the gene targets of Stat4 will be essential in ascertaining its role in myeloid cells. Although the function of Stat4 in murine myeloid cells is becoming clearer, its function in human myeloid cells is yet to be defined.

We demonstrate that upon activation, in Th1-dominated sites of inflammation, Stat4 is present in monocytes and available for phosphorylation by IFN-α. Although the role of Stat4 in the IL-12-dependent activation and differentiation of T cells and NK cells is well described, Stat4 expression in cells of monocyte lineage was previously unrecognized. Our data demonstrate that IFN-α signaling via Stat4 is a transduction pathway common to both lymphocytes and monocytes and complements the action of IL-12 signaling where IL-12 or its receptor are unavailable. In this manner, it may represent an important pathway in the early steps of the cellular immune response and is a potential target for therapeutic intervention.

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References


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