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*J Immunol* 2005; 175:6155-6162; doi: 10.4049/jimmunol.175.9.6155

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Selective Activation of Peripheral Blood T Cell Subsets by Endotoxin Infusion in Healthy Human Subjects Corresponds to Differential Chemokine Activation

Asit K. De,2* Carol L. Miller-Graziano,2,3* Steve E. Calvano,† Krzysztof Laudanski,* Stephen F. Lowry,† Lyle L. Moldawer,‡ Daniel G. Remick, Jr.,§ Natasa Rajicic,¶ David Schoenfeld,¶ and Ronald G. Tompkins¶

Although activation of human innate immunity after endotoxin administration is well established, in vivo endotoxin effects on human T cell responses are not well understood. Most naive human T cells do not express receptors for LPS, but can respond to endotoxin-induced mediators such as chemokines. In this study, we characterized the in vivo response of peripheral human T cell subsets to endotoxin infusion by assessing alterations in isolated T cells expressing different phenotypes, intracellular cytokines, and systemic chemokine concentration, which may influence these indirect T cell responses. Endotoxin administration to healthy subjects produced T cell activation as confirmed by a 20% increase in intracellular IL-2, as well as increased CD28 and IL-2R alpha-chain (CD25) expression. Endotoxin induced indirect activation of T cells was highly selective among the T cell subsets. Increased IL-2 production (36.0 ± 3.7 to 53.2 ± 4.1) vs decreased IFN-gamma production (33.8 ± 4.2 to 19.1 ± 3.2) indicated selective Th1 activation. Th2 produced IL-13 was minimally increased. Differentially altered chemokine receptor expression also indicated selective T cell subset activation and migration. CXCR3+ and CCR5+ expressing Th1 cells were decreased (CXCR3 13.9 to 1103.7, CCR5 24.8 to 12 ± 1.4), whereas plasma levels of their chemokine ligands IFN-gamma-inducible protein 10 and MIP-1a were increased (61.4 ± 13.9 to 1103.7 ± 274.5 and 22.8 ± 6.2 to 55.7 ± 9.5, respectively). In contrast, CCR4+ and CCR3 (Th2) proportions increased or remained unchanged whereas their ligands, eotaxin and the thymus and activation-regulated chemokine TARC, were unchanged. The data indicate selective activation among Th1 subpopulations, as well as differential Th1/Th2 activation, which is consistent with a selective induction of Th1 and Th2 chemokine ligands. The Journal of Immunology, 2005, 175: 6155–6162.

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Importantly, the ligands for these chemokine receptors can differentially activate their targets. CXCR3 agonists like IFN-γ-inducible protein 10 (IP-10) also act as CXCR3 antagonists preventing CCR3-induced migration (12). CCR7 is expressed on central memory T cells of both Th1 and Th2 types but these cells lack immediate effector functions and do not produce IFN-γ or IL-4 (9, 16). Conversely, CCR7-negative effector memory T cells express receptors for immediate migration to inflamed tissues and are capable of expressing both Th1 and Th2 cytokines (9, 16). Although the emigration of some T cells (transient reduction in peripheral T cell numbers) is an obvious consequence of endotoxin administration, selective migration patterns of peripheral T cell subsets in response to in vivo endotoxin might be secondary to selective chemokine induction. In this study, we have assessed T cell expression of chemokine activation receptors, induction of intracellular Th1- and Th2-type cytokine levels in purified T cells, and compared plasma chemokine levels in healthy subjects infected with endotoxin.

We demonstrate that specific subsets of human T cells respond differently to in vivo endotoxin administration. The presence of increased intracellular IL-2 induction as well as increased CD28 and IL-2R α-chain (CD25) expression in peripheral T cells suggested that these cells were activated after endotoxin administration. Activation of T cells was highly selective even among the Th0/Th1 population because IL-2 production was increased whereas IFN-γ production was decreased. Selective Th1 vs Th2 activation was paralleled by differential response of T cells with different chemokine receptor expression. The proportion of circulating CXCR3 and CCR5 expressing T cells (Th1) were reduced whereas the proportion of CCR4 and CCR3 expressing cells (Th2) were increased or unchanged. These T cell subpopulation alterations parallel a plasma increase in the CXCR3 ligand, IP-10, the CCR2 ligand, MCP-1, and the CCR5 ligand, MIP-1α. In striking contrast, the CCR3 ligand, eotaxin, and the CCR4 ligand, thymus and activation-regulated chemokine (TARC), were unaltered. Selective T cell emigration, in response to endotoxin infusion, was further suggested by an increasing numbers of CCR7-expressing central memory T cells in the face of generalized circulating T cell reduction. Thus, these data indicate a novel selective split activation of the human Th1 subsets in addition to differential Th1 vs Th2 activation as an indirect response to endotoxin-induced innate immunity and may reflect the novel differential chemokine activation also detected after endotoxin infusion.

Materials and Methods

Subjects

Seven adult male (age 28 ± 5.4 years) and two female (age 29 ± 5.4 years) subjects were recruited by public advertisement for entry into the protocol approved by the Institutional Review Board of University of Medicine and Dentistry of New Jersey (UMDNJ)-Robert Wood Johnson Medical School (New Brunswick, NJ). Written, informed consent was obtained before the performance of any study-related procedure. All subjects were in good general health as demonstrated by medical history, physical examination, and laboratory tests.

Human endotoxin model

On study day 1, the subject was admitted to the Clinical Research Center, UMDNJ-Robert Wood Johnson Medical School, and underwent a physical examination by 1500 h. The following morning, an i.v. catheter was placed in one upper extremity, and a continuous i.v. infusion of 5% dextrose in 0.45% sodium chloride (100 ml/h) was begun and continued until the subject tolerated a regular meal following completion of the acute phase of the study on the following day. In addition, the radial artery was cannulated percutaneously with a 20-gauge catheter. Vital signs and subjective symptoms of distress were monitored every 30 min for the next 6 h. Following baseline monitoring, subjects were administered National Institutes of Health Clinical Center Reference endotoxin (CC-RE-Lot 2) at a dose of 2 ng/kg body weight over a 5-min period through the i.v. catheter. Blood samples were collected before endotoxin infusion (0 h) and at post infusion times of 4, 9, and 24 h for plasma and T cells. Following collection of a 24-h blood sample, the subject was discharged. Differential peripheral blood white cell counts were taken at all of the time points.

Isolation of peripheral blood T cells

Peripheral blood (7 ml) was collected into one 7-ml EDTA containing Vacutainer tube. Human T cell enrichment mix (350 μl, RosetteSep® Stem-Cell Technologies) was added and incubated for 20 min at room temperature. The blood was diluted (1/1) with PBS containing 2% FBS and carefully layered on DM-L Ficoll (StemCell Technologies) and centrifuged for 20 min at 1200 × g at room temperature. The interfacial cells (T cells) were harvested, washed, and frozen with FBS/DMSO (90%/10% v/v) solution. The cells were stored for at least 24 h at −80°C before transporting the samples with dry ice to the University of Rochester School of Medicine.

Plasma collection

A second 7 ml of EDTA anticoagulated blood was centrifuged at 500 × g for 10 min at 4°C, plasma collected, then aliquoted and immediately frozen at −70°C. Frozen plasma samples were shipped to the University of Michigan for assay.

Flow cytometric analysis

Frozen T cells were thawed at 37°C, washed and suspended in RPMI 1640 supplemented with 2% FBS and antibiotics. The cell number and viability were determined by trypan blue exclusion using a hemocytometer. Cell viability ranged from 85 to 95%. For surface marker staining, 1 × 10^6 cells were labeled with pretitrated amounts of respective Abs or isotype controls. The surface markers tested were CCR2, CCR3 (R&D Systems), CCR4, CCR5, CCR7, CXCR3, CD28, CTLA4 (CD152), CD4, and CD25 (BD Pharmingen) in different four-color panels with CD2 as the T cell marker in each panel. CD2 was chosen as the T cell marker because CD3 is transiently down-regulated in activated T cells. The stained cells were washed and then analyzed in the BD FACSCalibur flow cytometer (BD Biosciences).

For assessment of intracellular cytokine production, T cells were activated with PMA (20 ng/ml) plus ionomycin (1 μM) for 10 h in presence of monensin (1 μM). The cells were then washed and stained first with CD2-biotin (followed by streptavidin-PerCP) as the T cell surface marker. The cells were washed and fixed with 200 μl of 1× Cytofix/Cytoperm reagent (BD Pharmingen) for 20 min at 4°C. The cells were then washed twice with 1× Perm/Wash solution (BD Pharmingen) and stained with Abs specific for intracellular cytokines such as IL-2, IL-4, IFN-γ, IL-10, and IL-13 or respective isotype controls (all purchased from BD Pharmingen) in three- or four-color panels and then analyzed in the flow cytometer.

Flow cytometric data are presented as 1) the percentage of positive cells; 2) mean fluorescent intensity (MFI) of the total T cells, calculated by subtraction of the fluorescent intensity of isotype control treated cells from the fluorescent intensity of the specific Ab-treated cells unless extremely low levels were obtained thus obviating subtraction (e.g., CD25 and IL-13); and 3) mean cell intensity (MCI) defined as MFI of only the T cell population positive for a specific surface, or intracellular cytokine marker (17). A reduction in the percentage of positive cells expressing a particular receptor could represent transient migration from the circulation, down-regulation of the receptors being assessed, or both. Assessing MCI as well as MFI and the percentage of positive cells allows discrimination between a reduced receptor density due to a reduction in the total cells expressing the receptor (MFI) vs a reduction of the actual receptor density on those cells still present in the circulation. A decrease in the percentage of positive cells and MFI of the total T cell population, but no decrease in the MCI of the remaining T cells that were expressing the receptor, would imply that the overall number of cells expressing the receptor was decreased. However, the receptor on the remaining cells was expressed at comparable levels (no down-regulation) to untreated cells in the peripheral circulation. Conversely, a change in all three measures of marker expression (the percentage of positive cells, MFI, and MCI) would indicate activation, migration, and altered receptor expression.

*Abbreviations used in this paper: IP-10, IFN-γ-inducible protein 10; MFI, mean fluorescent intensity; MCI, mean cell intensity; TARC, thymus and activation-regulated chemokine.*
Chemokine analysis

Chemokines were measured using the bead array assay kit from Linco Research, which measures 22 cytokines in a multiplex format. The concentrations of the individual analytes were determined using the MiraiBio software. TARC was assessed using a specific ELISA.

Statistical analysis

Data are presented as mean plus SEM. A two-way ANOVA was used to test whether each of the measured markers varied over time. When this measurement was significant, the difference between each time point and the 0 h baseline was assessed using both ANOVA and Student’s t test for paired samples. A two-tailed hypothesis was considered and the differences between time points were considered statistically significant if \( p < 0.05 \). There were no corrections for multiple comparisons.

Results

Effects of endotoxin administration on peripheral T cell counts

Although most human T cells lack receptors for LPS, they are still activated during innate immunity by cytokines produced in response to endotoxin exposure (6, 18). Administration of endotoxin induced a significant reduction in total peripheral lymphocyte numbers (expressed as a percentage) in Fig. 1a and in total numbers in Fig. 1b at 4 and 9 h postinfusion, followed by a return to approximately normal levels at 24 h. Transient reduction in peripheral T cell numbers in response to endotoxin exposure could occur secondary to a net emigration of one or more subsets of T cells out of the active circulation and into high endothelial vessels (12). Phenotypic characterization of the T cells remaining in the peripheral blood would describe the indirect effect of endotoxin-induced innate immunity on activation and/or migration of different T cell subsets.

In vivo LPS induces activation of T cell subsets

T cell subsets are often identified by their cytokine production. Interaction of Ag-bound APCs with T cells leads to their activation, as reflected by increased IL-2 production, an increased number of cells expressing the IL-2R α-chain (CD25) and by up-regulation of the costimulatory molecule CD28, which is critical for appropriate T cell function (19, 20). Indirect activation of T cells by endotoxin-induced innate immunity should also be reflected by changes in some of the same receptors and cytokines. Therefore, we first examined the in vivo effects of endotoxin administration on T cell expression of CD28, CD25, and IL-2. Although a majority of control T cells (data at 0 h) expressed CD28 at baseline, there was still a significant increase in CD28 expression (as determined by the percentage of positive cells, their mean MFI as well as their mean MCI levels), following endotoxin administration (Fig. 2a). Activation of T cells was also suggested by an increase in the number and expression of CD25+ T cells (Fig. 2b) and the intracellular expression of IL-2 (Fig. 2c).

Endotoxin administration differentially activates T cell subsets as indicated by altered cytokine production patterns

Although in vivo endotoxin administration induced an overall T cell activation response, individual T cell subsets may have responded differentially. Therefore, we evaluated the expression of IL-2.

- **FIGURE 1.** In vivo endotoxin administration reduces overall lymphocyte percentage and absolute T cell numbers in human peripheral blood. a, Percentage of lymphocytes from peripheral blood isolated from normal volunteers at indicated time points after endotoxin administration, presented as the percentage of positive cells. b, T cells were isolated from 7 ml of peripheral blood simultaneous to differential counting at indicated time points and counted for assessment for absolute T cell recovery. *, \( p < 0.05 \) and **, \( p < 0.01 \) and ***, \( p < 0.001 \) as compared with 0 h data (\( n = 9 \)).

- **FIGURE 2.** Activation of peripheral T cells by in vivo endotoxin administration. Peripheral T cells isolated at indicated time points after endotoxin administration were assessed for surface expression of CD28 (a) and CD25 (b). c, T cells were also stimulated ex vivo with PMA (20 ng/ml) and ionomycin (1 μM) for 10 h in the presence of monensin (1 μM), then assessed for intracellular expression of IL-2 by flow cytometry. Data are presented as the percentage of positive cells, net MFI, as well as MCI. *, \( p < 0.05 \) and **, \( p < 0.01 \) as compared with 0 h data (\( n = 9 \) for CD28 and IL-2; and \( n = 6 \) for CD25).
specific T cell receptors and cytokines associated with different T cell subsets. Recently, the expansion of CD4\(^+\)CD25\(^+\) regulatory T cells (normally 3–6% of peripheral T cells) with inhibitory activity in peripheral blood has been characterized in many diseases (21, 22). Because CD25 expression was increased in T cells following endotoxin administration, we examined the selective expansion of such regulatory T cell populations in the circulation. There was indeed an increase in the percentages of CD4\(^+\)CD25\(^+\) T cells following endotoxin administration, we examined the selective expansion of such regulatory T cell populations in the circulation. There was indeed an increase in the percentages of CD4\(^+\)CD25\(^+\) T cells following endotoxin administration (Fig. 3, a–d). However, this increase in CD4\(^+\)CD25\(^+\) T cell numbers was not accompanied by any increase in surface expression of CTLA4, both a marker for and a molecule active in negative signaling in regulatory T cells (data not shown). CTLA4 expression was <2% at all time points up to 24 h following endotoxin administration. Thus, increases in CD25 expression following endotoxin administration may only reflect activation of T cells rather than any selective retention and/or infiltration of regulatory T cells, at least during the 24-h assay period.

Subsequently, we tested the expression of the Th1-specific cytokine IFN-\(\gamma\) because this cytokine is reported as being downregulated in the whole blood from endotoxin-administered volunteers (23). Similar to the whole blood data, IFN-\(\gamma\) production by purified T cells was significantly reduced at 4 h after endotoxin administration (Fig. 3e). In contrast to reduced IFN-\(\gamma\)-producing
Th1 cells, the number of IL-13-producing Th2 cells was modestly increased at 4 and 9 h (Fig. 3f). However, T cell IL-10 production was unchanged (<2% at all time points) after endotoxin administration (data not shown).

Although a shift in Th1 vs Th2 cytokine levels has been characterized in the whole blood response to endotoxin, the mechanisms underlying this shift as well as any direct differential activation within the Th2, Th1, and/or Th0 subsets remains uncharacterized. Therefore, we simultaneously assessed the intracellular IL-2 and IFN-γ levels (both produced by Th1 and Th0 cells) in the same isolated T cells following endotoxin administration. Surprisingly, single IL-2-producing T cell numbers were increased whereas single IFN-γ-producing T cells were reduced following endotoxin exposure (Fig. 4). However, T cells producing both IL-2 and IFN-γ were initially slightly reduced at 4 h, but again increased at 9 h post endotoxin administration (Fig. 4e). These data suggest a previously unrecognized effect of in vivo endotoxin-induced response in differential activation of T cell subsets even within the Th1 (and Th0) population, in addition to selective activation effects on Th1 vs Th2 populations.

**In vivo endotoxin infusion induces differential T cell subset activation and migration as reflected by alterations in chemokine receptor expression in residual T cell populations**

We subsequently characterized the effect of endotoxin-induced innate immunity on different T cell subsets expressing distinct chemokine receptors that would indicate their migratory capacity as well as their Th1/Th2 phenotype. Cells expressing CCR5 (expressed on Th1 memory cells associated with migration to inflammatory sites) were significantly reduced at 4 and 9 h, both in the percentage of positive cell numbers and mean MFI levels (Fig. 5a). However, MCI levels of CCR5+ cells were only modestly reduced, suggesting that the level of receptor expression on the positive cells was unaffected. One possible explanation for this result is a selective migration of this inflammation related Th1 subset from the circulation. Expression of CXCR3 (also expressed on memory Th1 cells) was significantly reduced, as determined by the percentage of positive cells and MFI and MCI levels, indicating overall down-regulation of CXCR3 on resident Th1 cells in addition to their migration (Fig. 5b). Although the expression of CXCR2 was low in the control T cell population (data at 0 h), the T cell CXCR2 expression was further significantly reduced at 4 h as shown in the percentage of positive numbers while MCI was unaffected, further suggesting a selective migration of Th1-associated T cells from the circulation (Fig. 5c). Reduced numbers of circulating T cells expressing CCR5 and CXCR3 concomitant to increases in circulating CCR4 expressing T cell numbers (predominantly Th2 cells) has also been seen in Crohn’s disease and was associated with inflammatory Th1 cell activation and migration to the gut (24). We, therefore, assessed the level of T cells expressing CCR4 in the face of endotoxin-induced transient inflammatory responses. In fact, the number of CCR4-expressing T cells was significantly increased at 4–9 h, as measured by the percentage of positive cells and by MFI and MCI levels, indicating selective retention of CCR4+ T cells in the circulation as well as overall up-regulation of CCR4 expression (Fig. 6a). However, there was minimal increase in T cells expressing (<2% at all time points) the Th2-associated marker CR3, indicating that these highly activated Th2 cells were primarily unaffected (Table I). Finally, we examined whether in vivo endotoxin administration affected T cell CCR7 expression because CCR7+ T cells are reported as lacking effector functions and incapable of IFN-γ or IL-4 production but capable of IL-2 production (9, 16). The number of T cells expressing CCR7 was increased at 4 h shown both in the percentage of positive cell numbers and their MFI levels, but MCI levels were unchanged. These data strongly suggest selective retention of CCR7+ T cells in the peripheral circulation after endotoxin administration (Fig. 6b).

**Selective activation/migration of T lymphocyte subsets parallels differential plasma chemokine levels**

Circulating human monocytes, NK, and B cells all express LPS responsive receptors and can respond to endotoxin infusion with chemokine production (12). Subsequently, endotoxin-insensitive human T cells can respond to these chemokines by specific migration, as well as activation (12). The plasma levels of IP-10 (CXCR3 ligand), MCP-1 (CCR2 ligand), eotaxin (CCR3 ligand), TARC (CCR4 ligand), and MIP-1α (CCR5 ligand) were assessed because these chemokines are produced in response to pathogens and can differentially affect the activation/migration of the T cell subsets measured (12). Increases in RANTES cannot be assessed in plasma because of existing high plasma levels (12). As seen in Table I, IP-10, MCP-1, and MIP-1α were significantly increased whereas eotaxin and TARC were generally unchanged. TARC levels were highly variable between individuals. Increases in plasma MIP-1α paralleled the decreased circulating numbers of T cells expressing CCR5 (its receptor) whereas increases in IP-10 paralleled decreases in T cells expressing CXCR3. Eotaxin levels were unchanged as were its receptor-expressing T cells (CCR3). The increase in circulating numbers of CCR4 expressing T cells corresponded to overall unaltered levels of the CCR4 ligand TARC.

**FIGURE 5.** Decrease in Th1-specific chemokine receptor expression after in vivo endotoxin administration. Peripheral T cells isolated at indicated time points after endotoxin administration were assessed for surface expression of different chemokine receptors such as CCR5 (a), CXCR3 (b), and CCR2 (c) by flow cytometry. Data are presented as the percentage of positive cells, net MFI, as well as MCI. *, p < 0.05; **, p < 0.01, and ***, p < 0.0001 as compared with 0 h data (n = 9 for CCR5 and CXCR3; n = 6 for CCR2).
monocytes, all of which can produce different Th1- or Th2-type cytokines. In this study, we report that endotoxin administration to healthy humans elicits a discordant effect on the activation and emigration of circulating Th1 and Th2 cells based on subset-specific intracellular cytokine production and chemokine receptor expression of isolated T cells. In addition, our data further extend the characterization of in vivo endotoxin-induced selective T cell activation within the Th1 cell populations themselves and correlate these changes in T cell subsets with increases of the corresponding plasma chemokinases. We demonstrate for the first time that endotoxin-stimulated innate immunity induces not only differential activation of human Th1 and Th2 cells, but also variable induction of Th1/Th2 cytokines (↑ IL-2 but ↓ IFN-γ) and Th1/Th0 migration as well as differential chemokine levels. Isolated T cell cytokine levels were directly assessed. Consequently, any contribution of other cell types to cytokine levels, as might appear in whole blood experiments, was eliminated. This result may account for our detection of split T cell production of IL-2 and IFN-γ in contrast to whole blood studies (23).

The simultaneous examination of intracellular cytokine production and chemokine receptors suggests that some T cells experience both dramatically altered receptor expression and selective migration out of the active circulatory population (as indicated by a reduction in peripheral T cell numbers after endotoxin administration). Selective migration of T cell subsets has been reported in many disease states (24, 27). In addition, activation through CCR5 has been shown to greatly increase T cell/endothelial cell adherence that could explain why the MIP-1α-activated (CCR5 ligand) T cells appear depleted from the circulating T cell pool (28). The selective migration after in vivo endotoxin appears to correspond to the selective activation of specific chemokines. A recent report suggested that CXCR3 and CCR4 expression identifies memory pre-Th1 and pre-Th2 cells, respectively, that become fully differentiated Th1 and Th2 effector cells in response to cytokines alone (IL-7 and IL-15) (11). It is possible, therefore, that some of the activated (CCR4) Th2 cells we see are also of the memory populations, which are ready to respond to endotoxin-induced cytokines. However, the data can also be explained by an increase in the proportion of CCR4+ cells due to selective migration and adherence of other Th1 cell subsets to high endothelial vessels in response to endotoxin-induced increases in selective chemokines for which these subsets express receptors (12). Failure of in vivo endotoxin infusion to induce chemokines, which are CCR4 and

Discussion

Activation and expansion of Th1 and Th2 cells in response to different stimuli have been well characterized (25, 26). Differential activation of Th1 and Th2 cells as indicated by cytokine production in response to in vivo endotoxin administration has also been suggested on the basis of data obtained from whole blood experiments (23). However, whole blood responses represent cytokines made by all leukocytes including NK cells, B cells, mast cells, and monocytes, all of which can produce different Th1- or Th2-type cytokines. In this study, we report that endotoxin administration to healthy humans elicits a discordant effect on the activation and emigration of circulating Th1 and Th2 cells based on subset-specific intracellular cytokine production and chemokine receptor expression of isolated T cells. In addition, our data further extend the characterization of in vivo endotoxin-induced selective T cell activation within the Th1 cell populations themselves and correlate these changes in T cell subsets with increases of the corresponding plasma chemokinases. We demonstrate for the first time that endotoxin-stimulated innate immunity induces not only differential activation of human Th1 and Th2 cells, but also variable induction of Th1/Th2 cytokines (↑ IL-2 but ↓ IFN-γ) and Th1/Th0 migration as well as differential chemokine levels. Isolated T cell cytokine levels were directly assessed. Consequently, any contribution of other cell types to cytokine levels, as might appear in whole blood experiments, was eliminated. This result may account for our detection of split T cell production of IL-2 and IFN-γ in contrast to whole blood studies (23).

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Table 1. Increased plasma chemokine ligands of Th0/Th1 cells following LPS administration parallel migration/depletion of T cells expressing the reciprocal receptor

<table>
<thead>
<tr>
<th>Chemokine&lt;sup&gt;a&lt;/sup&gt;</th>
<th>0 h</th>
<th>4 h</th>
<th>9 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>TARC (ligand for CCR4)</td>
<td>256.5 ± 64.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>260.2 ± 53.6</td>
<td>309.4 ± 64.3</td>
<td>315.9 ± 87.1</td>
</tr>
<tr>
<td>EOTAXIN (ligand for CCR3)</td>
<td>57.6 ± 8.4</td>
<td>49.4 ± 13</td>
<td>41.5 ± 14.1</td>
<td>70 ± 14.9</td>
</tr>
<tr>
<td>IP-10 (ligand for CXCR3)</td>
<td>61.4 ± 13.9</td>
<td>1103.7 ± 274.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>278.5 ± 81.1</td>
<td>114.8 ± 30.3</td>
</tr>
<tr>
<td>MCP-1 (ligand for CCR2)</td>
<td>59.4 ± 10.4</td>
<td>1481.2 ± 202.4&lt;sup&gt;**&lt;/sup&gt;</td>
<td>129.5 ± 20.7&lt;sup&gt;*&lt;/sup&gt;</td>
<td>61.5 ± 13.7</td>
</tr>
<tr>
<td>MIP-1-α (ligand for CCR5)</td>
<td>22.8 ± 6.18</td>
<td>55.7 ± 9.5&lt;sup&gt;*&lt;/sup&gt;</td>
<td>25.7 ± 4.2</td>
<td>24.4 ± 5.9</td>
</tr>
</tbody>
</table>

Chemokine receptor expression<sup>d</sup>

| CCR4 | 13.28 ± 1.28<sup>e</sup> | 15.97 ± 1.1<sup>*</sup> | 20.86 ± 1.83<sup>**</sup> | 15.15 ± 1.67 |
| CCR3 | 1.54 ± 0.19 | 1.82 ± 0.34 | 1.70 ± 0.13 | 1.65 ± 0.2 |
| CXCR3 | 44.57 ± 3.17 | 30.26 ± 4.58<sup>**</sup> | 40.17 ± 3.94 | 40.42 ± 3.76 |
| CCR2 | 3.66 ± 1.04 | 1.86 ± 0.2<sup>*</sup> | 2.7 ± 0.55 | 3.2 ± 0.51 |
| CCR5 | 24.83 ± 2.34 | 12.01 ± 1.42<sup>***</sup> | 18.87 ± 2.54<sup>**</sup> | 23.17 ± 2.3 |

<sup>a</sup> Chemokines were measured in the plasma by Luminex assay or ELISA and expressed as picograms per milliliter.
<sup>b</sup> Values in the “Chemokine” section are concentrations (mean ± SEM) in picograms per milliliter at times post-LPS administration.
<sup>c</sup> *, p < 0.05; **, p < 0.01; and ***, p < 0.0001 as compared to 0 h data (n = 6 for chemokine levels, CCR2, CCR3, TARC, and CCR4; n = 9 for CCR5 and CXCR3).
<sup>d</sup> T cells were stained with respective chemokine receptors or isotype controls as described in Materials and Methods and expressed as the percentage of positive cells.
<sup>e</sup> Values in the “Chemokine receptor expression” section are shown as percent-positive (mean ± SEM).
CCR3 ligands, would result in selective retention of T cell subsets expressing these receptors. Some of the CXCR3 (Th1) memory cells are also induced to express CCR5 (the receptor for inflammatory chemokines like MIP-α and MIP-β as well as RANTES), which then results in their migration to inflammatory sites. The decrease in CCR5 and CXCR3 expressing T cells after in vivo endotoxin administration might also be a result of endotoxin-induced chemokine stimulation of this memory CXCR3 Th1 population by MIP-1α. Enhancement of circulating CCR4 and diminution of CCR5 and CXCR3 expressing T cells also parallels the characteristic chemokine receptor profile alterations in peripheral blood memory T cells seen in Crohn’s disease where Th1 cells preferentially migrate to sites of intestinal inflammation (24). In contrast, CCR5 and CXCR3 expressing Th1 cells increase in peripheral blood populations in multiple sclerosis (a disease in which circulating T cells produce higher levels of IFN-γ) (27). Our data also suggest preferential migration of Th1 cells concomitant to selective circulatory retention of Th2 cells following in vivo administration of endotoxin and selective activation of the chemokine ligands for these Th1 and Th2 chemokine receptors. In addition, our data indicate selective migration of CCR7-negative effector memory T cells with preferential retention of CCR7-positive central memory T cells. Unfortunately, we did not assess the CCR7 ligands MIP-3β or 6Ckine levels in the plasma. Selective migration of CCR7-negative effector memory T cells may also partially explain the decreased T cell IFN-γ production and increased IL-2 production because CCR7+ T cells lack effector functions and are incapable of IFN-γ production but can produce IL-2 (9, 16). Although the possibility of selective retention of regulatory T cells following endotoxin administration was not supported by our data, we cannot completely eliminate this possibility because of the slow induction of surface expression of CTLA4, and its preferential intracellular expression, which was not assessed in this study. Moreover, selective retention of regulatory T cells would have been more appropriately indicated by increased expression of the regulatory T cell-specific cytokine TGF-β and/or expression of the transcription factor, FoxP3 (29). In vivo endotoxin exposure might also differentially activate CD4 vs CD8 memory and effector populations. All these possible effects of in vivo endotoxin-induced innate immunity on differential activation of human T cells remain to be explored.

Recently, interactions between innate and adaptive immunity have become the focus of considerable investigation. However, most of these studies have been performed in murine models, and there exist significant differences in the innate and adaptive immune interactions between humans and mice, particularly in TLR expression and monocyte activation (30). In vivo studies in humans, although extremely difficult to undertake, can more clearly reveal the nature of those unique human immune interactions. The in vivo infusion of endotoxin into healthy human subjects is an excellent model for studying such relationships independent of patient comorbidities or treatment intervention variables. The data in this study are a novel indication of, first, differential induction of human chemokines in response to in vivo endotoxin infusion and, second, that these chemokines could be mediating the increase in circulating Th2 lymphocytes by selectively mediating the migration of not just Th1/Th0 lymphocytes but also of specific Th1 subsets. The failure to see major increases in IL-13 and IL-10 further support that selective retention rather than proliferation of Th2-type cells is occurring. Although elevated plasma levels of IL-10 have been detected after endotoxin infusion, this IL-10 may result from direct endotoxin activation and IL-10 production by human B cells, not from Th2 cells (31). The rapid and selective migration response of human Th1 lymphocytes after Gram-negative bacterial activation of the innate immune system may contribute to the highly Th1 preferential responses seen in most human pathogen stimulation systems.

Acknowledgments

We thank the technical and support staff who contributed to the successful completion of these studies. In particular, we thank Leanne Staples and Joyce Kriege. Additional participating investigators in the Large Scale Collaborative Research Agreement entitled, “Inflammation and the Host Response to Trauma” include Drs. Henry V. Baker, Paul E. Bankey, Timothy R. Billiar, Bernard H. Brownstein, David Camp II, Celeste Campbell-Finnerty, George Casella, Ishsad H. Chaudry, Maskhoor Choudhry, J. Perren Cobb, Ronald W. Davis, Constance Elson, Bradley Freeman, Richard L. Gamelli, Nicole S. Gibran, Douglas L. Hayden, Drs. Brian G. Harbrecht, David N. Herndon, Jureta W. Horton, William Hubbard, John Lee Hunt, Jeffrey Johnson, Matthew B. Klein, James A. Lederer, Tanya Logvinenko, Ronald V. Maier, John A. Mannick, Philip H. Mason, Grace P. McDonald-Smith, Bruce A. McKinley, Michael N. Mindrinos, Joseph P. Minei, Ernest E. Moore, Frederick A. Moore, Avery B. Nathens, Grant E. O’Keeffe, Laurence G. Rahme, Michael B. Shapiro, Martin Schwacha, Geoffrey M. Silver, Richard D. Smith, John Storey, Mehmet Toner, H. Shaw Warren, Michael A. West, and Wenzhong Xiao.

Disclosures

The authors have no financial interest of interest.

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