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Stimulation of Human T Lymphocytes by LPS Is MHC Unrestricted, But Strongly Dependent on B7 Interactions

Taila Mattern,* Hans-Dieter Flad,* Lore Brade,† Ernst T. Rietschel,† and Artur J. Ulmer2*  

Recently, we have shown that LPS is a potent inducer of human T cell proliferation and lymphokine production. However, the activation of T cells by LPS has been demonstrated to be monocyte dependent and to require direct cell-to-cell contact. Here, we investigated the role of monocytes as accessory cells and the requirement for costimulatory signals in more detail. We found that the accessory cell activity of monocytes during LPS-induced T cell proliferation is characterized by the following features: LPS-primed monocytes are competent stimulators of T cell proliferation; interaction of LPS with monocytes during the priming step is dependent on CD14 and is sensitive to ammonia; monocyte/T cell interactions are not MHC restricted but are strongly dependent on interactions of CD28 and/or CTLA-4 on T cells and their ligands CD80 and/or CD86 on monocytes. CD80 seems to be crucial for the activation of T cells by monocytes, since monocytes expressing CD86 but not CD80 after LPS stimulation were unable to stimulate T cells; IL-12, at least as a costimulatory factor, but not IL-15, is important in LPS-induced T cell proliferation. Taken together, our results indicate that LPS acts neither as a mitogen, nor as a superantigen, nor as an Ag. The activation of human T cells by LPS requires the help of accessory functions by primed monocytes and is MHC unrestricted but needs co-stimulatory signals via CD28 and/or CTLA-4. The Journal of Immunology, 1998, 160: 3412–3418.

A large diversity of different cell types has been described that can be activated by LPS of Gram-negative bacteria (reviewed in Refs. 1 and 2). Activation of human or murine monocytes/macrophages is a well-established property of LPS and its biologically active component lipid A. The activation of murine B lymphocytes and murine T lymphocytes is also well documented (3–9). However, less known are the effects of LPS on human T lymphocytes (10, 11). Recently, we have shown that LPS and its lipid A component are potent inducers of human T cell proliferation and of Th1-like lymphokines (12). Furthermore, we found that stimulation of T cells by LPS requires the presence of viable monocytes, which could not be replaced by p-formaldehyde-fixed monocytes. Additionally, neither a mixture of monokines nor a combination of fixed monocytes and monokines was able to support T cell proliferation (12, 13). This indicated that T cell stimulation is strongly dependent on direct cell-to-cell contact between T lymphocytes and accessory monocytes.

The aim of this study was to define the role of monocytes and accessory costimulatory signals in more detail. We investigated the role of CD14/LPS interactions, the effects of cytosomotropic agents, MHC restriction, and the role of costimulatory signals via CD28 and/or CTLA-4. Additionally, we examined whether B7.1 (CD80) or B7.2 (CD86) are differentially up-regulated on monocytes from LPS responders and nonresponders. We also investigated the role of IL-12 and IL-15 during the LPS-induced T cell proliferation. It will be shown that the accessory cell activity of monocytes during LPS-induced T cell proliferation is dependent on CD14/LPS interactions and is sensitive to ammonia. The T cell/monocyte interaction during LPS-induced T cell proliferation is MHC unrestricted and strongly dependent on costimulatory signals through CD28/CTLA-4. We demonstrate that the CD28/CTLA-4 ligand CD80 is crucial for LPS-induced T cell proliferation since it is induced by LPS on monocytes of LPS responders, but not of LPS nonresponders. Furthermore, we show that addition of anti-IL-12 mAb but not anti-IL-15 mAb inhibit the LPS-induced T cell proliferation.

Materials and Methods

Bacterial mitogens

LPS of Salmonella friedenau and Salmonella minnesota R7 was prepared by the phenol-water method as described (14), purified by repeated ultra-centrifugation, and converted to the sodium salt after electrodialysis, as reported previously (15). Such preparations contain <0.1% of protein and nucleic acid, as determined by chemical analysis.

Azide-free tetanus toxoid (TT)† was obtained from Behringwerke AG, Marburg, Germany, and azide-free purified protein derivatives of Mycobacterium tuberculosi (PPD) was obtained from Statens Serum Institut, Copenhagen, Denmark. The superantigen staphylococcal enterotoxin B (SEB) was obtained from Sigma, Munich, Germany.

Isolation of cells

PBMC were isolated from heparinized blood of healthy human donors by density centrifugation, as described by Böyum (16). Purified monocytes and lymphocytes were isolated from PBMC by counterflow centrifugation using a Beckman JE-6B ultracentrifuge (Beckman Instruments, Munich, Germany). The monocyte fraction collected consisted of >95% monocytes, as determined by FACS analysis after staining with anti-CD14 (Leu-M3, Becton Dickinson, Heidelberg, Germany). T cells were isolated from the eluted lymphocyte fraction by nylon wool filtration (nylon wool fiber, Polysciences, Eppelheim, Germany) according to the methods described by Julius et al. (17). The nonadherent cells consisted of >95% of CD2+ T lymphocytes (Leu-5b, Becton Dickinson). The concentration of monocytes within these purified T cells was below the detection limit (<0.1%, as determined by chemiluminescence) (18). The content of B cells was 0.5 to 3 Abbreviations used in this paper: TT, tetanus toxoid; HS, human serum; PPD, purified protein derivatives of M. tuberculosis; SEB, staphylococcal enterotoxin B.

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3%, as determined by FACS analysis after staining with anti-CD20 (Leu-16, Becton Dickinson).

Cell culture

Purified monocytes were primed with LPS from *S. friedenau* (1 μg/ml), TT (1 times Florence dose (Lf)/ml), or SEB (10 ng/ml) by incubation in RPMI 1640, supplemented with 10% heat-inactivated human serum (HS) and antibiotics for 1 h at 37°C. Monocytes were then washed intensively and added to autologous or heterologous T cells (1 × 10^6/ml) at a final concentration of 1 × 10^6/ml. They were cultured in flat-bottom plates (Nunc, Roskilde, Denmark) at a final volume of 200 μl. Cells were cultured for 4 days, if stimulated with SEB, or for 7 days in case of LPS- or TT-induced proliferation. For the last 8 h of stimulation, cells were labeled with [^3]H]Tdr (sp. act. 2 Ci/mmol, 0.2 μCi/ml, Amersham Buchler, Braunschweig, Germany) and harvested on glass-fiber filters for measurement of incorporated radioactivity.

Binding studies with LPS-primed monocytes

For these studies, purified monocytes were treated with LPS from *S. minnesota* R7 (1 μg/ml) for different time intervals. One part of these primed monocytes (1 × 10^6/ml) was added to purified autologous T lymphocytes (1 × 10^6/ml) to determine their capacity to induce T cell proliferation. Another portion of primed monocytes was labeled with an anti-S. minnesota LPS mAb as described below to detect membrane-bound LPS.

Inhibition of LPS priming by anti-CD14 mAb

In some experiments, purified monocytes were incubated for 1 h at 4°C with the azide-free anti-CD14 mAb MEM-18 (7 μg/ml, a kind gift of V. Hołejś, Czechoslovak Academy of Sciences, Prague, Czech Republic) (19) and then washed twice over a FCS gradient. Thereafter, monocytes were treated for 1 h at 37°C with the stimuli and washed again twice over a FCS gradient. The culture medium in these experiments was RPMI 1640, supplemented with 10% HS. Primed monocytes (1 × 10^6/ml) were added to purified T cells (1 × 10^6/ml) and cultured for 7 days. Then, Tdr incorporation was measured as described above.

Treatment of monocytes with ammonium chloride

Purified monocytes (2 × 10^6/ml, 1 ml/glass tube) were incubated with ammonium chloride (NH₄Cl, Merck, Darmstadt, Germany) in concentrations as indicated in the figure legends for 30 min at 37°C in serum-free RPMI 1640. The stimuli (LPS 2 μg/ml, SEB 20 ng/ml, TT 2 Lf/ml) were added in 1 ml RPMI 1640, supplemented with 20% HS. Cells were incubated at 37°C for further 2 h and then washed intensively. Of these pretreated monocytes (2 × 10^6/ml) 100 μl were added to 100 μl autologous T lymphocytes (2 × 10^6/ml) and cultured for 4 days (SEB-stimulated cultures), or 7 days (LPS- or TT-stimulated cultures), respectively. DNA synthesis was measured as described above.

Studies with anti-MHC class II mAb, CTLA-4 Ig, anti-IL-12 mAb, or anti-IL-15 mAb

For blocking experiments, PBMC (1 × 10^6/ml) were stimulated with LPS (1 μg/ml), TT (1 Lf/ml), PPD (10 μg/ml), or SEB (10 ng/ml) in the presence of 10 μg/ml anti-MHC class II mAb (anti-HLA-DR, -DQ, and -DR, clone Ti29, Pharmingen, Hamburg, Germany), the chimeric protein of human CTLA-4 and murine Ig (CTLA-4 Ig, a kind gift of Dr. P. Lane, Basel Institute, Basel, Switzerland), blocking anti-IL-12 mAb (10 μg/ml, clone C8.6, Endogen, Woburn, MA), or blocking anti-IL-15 mAb (10 μg/ml, clone M111, Genzyme, Cambridge, MA). The final concentrations of CTLA-4 Ig are indicated in the figure legends.

Induction of CD80/CD86

PBMC (1 × 10^6/ml, 1 ml/well) were cultured in 24-well culture plates (Nunc) for 48 h in the presence or absence of LPS (1 μg/ml) in RPMI 1640, supplemented with 10% heat-inactivated HS. Cells were collected after careful rubbing, to yield all adherent monocytes. Cells were then stained with mAb as described below.

Immunofluorescence staining of cells

The following mAb were obtained from Pharmingen, Hamburg, Germany: anti-CD86-biotin (anti-B7.2, clone Ti2.2, IgG2b) and isotype controls IgG2b-FTTC and IgM-FTTC. Anti-CD80-biotin (anti-B7.1, clone BB.1, IgM) was obtained from Ancell, Bayport, MN, and streptavidin-Red670 was obtained from Life Technologies, Berlin, Germany. The anti-LPS Ab S3232 (IgG2a) is specific for the sugar core of LPS from *S. minnesota* R7 (20). Indirect immunofluorescence staining of PBMC was performed in ice-cold PBS (containing 0.1% sodium azide) with the different Ab in concentrations as recommended by the producers. S3232 was used in a 1:25 (culture supernatant) dilution. After incubation for 20 min at 4°C, cells were washed by centrifugation on a FCS gradient (200 × g for 10 min). Biotinylated antibodies were detected by further incubation for 20 min with streptavidin-Red670. Unbound streptavidin-Red 670 was again removed by centrifugation over a FCS gradient. Labeled cells were analyzed in a Cytofluorograf (System 50H, Ortho Diagnostic Systems, Westwood, MA).

Results

T cell proliferation in the presence of primed monocytes

As described in a previous study, LPS-primed monocytes are able to stimulate the proliferation of human T lymphocytes (12). In first experiments of the present study, the time-kinetics of priming monocytes by LPS were analyzed. Monocytes were incubated for 5, 20, 60, or 120 min with LPS of *S. minnesota* R7. After this pretreatment, unbound LPS was removed by washing the monocytes. These primed monocytes were used as stimulatory cells for autologous T lymphocytes. In addition, we investigated the presence of membrane-bound LPS on the primed cells with a LPS-specific mAb. As a control for the stimulatory capacity of primed monocytes, the proliferative response of T cells in unseparated PBMC was measured. In these cultures with PBMC, the LPS was present during the entire culture period. Typical results are shown in Figure 1. With increasing time of priming, the number of surface-bound LPS, as well as the stimulatory capacity of primed monocytes, increased and reached optimal levels after ~60 min of preincubation. Interestingly, only monocytes with saturated LPS-binding sites were able to induce optimal T cell proliferation. In additional experiments, therefore, monocytes were primed for 1 h with LPS.

The role of CD14 during priming of monocytes

CD14 is a well-documented LPS receptor on monocytes. To investigate whether CD14 also acts as a LPS receptor during priming of monocytes, we examined the effects of anti-CD14 mAb. For this purpose, monocytes were first preincubated with anti-CD14 mAb and then primed with LPS, TT, or SEB as described in Materials and Methods. These primed monocytes were added to purified T cells, and [^3]H]Tdr incorporation was determined after 4 days (with SEB-primed monocytes) or 7 days (TT- or LPS-primed monocytes, respectively) of culture. Typical results of such experiments are shown in Table I. Pretreatment of monocytes with anti-CD14 mAb resulted in a significant inhibition of T cell proliferation induced by LPS-primed monocytes, whereas stimulation of T cells with TT- or SEB-primed monocytes was not affected.

Effects of ammonium chloride during priming

Next, we investigated the effects of the lysosomotropic agent ammonium chloride. In these experiments, priming of monocytes by the different stimuli was performed in the presence of different concentrations of ammonia. Typical results of such experiments are shown in Figure 2. While stimulation of T lymphocytes with the superantigen SEB was not affected by any concentration of ammonia tested, there was a clear dose-dependent reduction in T cell proliferation induced by the Ag TT. Interestingly, LPS-induced T cell proliferation was also inhibited by ammonia at a dose effect comparable with the inhibition of Ag-driven proliferation. This finding indicates that the stimulatory effect of LPS is sensitive to inhibition by ammonia.
Induction of T cell proliferation in the presence of primed heterologous monocytes

Next we investigated whether or not the interaction of LPS-primed monocytes and T cells was MHC restricted. We compared the stimulatory activity of primed autologous as well as primed heterologous monocytes. Monocytes and T lymphocytes were isolated from two different donors, and T cells were stimulated with LPS-, TT-, or SEB-primed monocytes, respectively. As a control, [3H]TdR incorporation in unstimulated cultures was determined. The results of these experiments are shown in Table II. As expected, [3H]TdR incorporation of T cells stimulated with TT-primed monocytes was slightly over background levels, indicating MHC restriction in antigenic T cell stimulation. On the other hand, SEB-primed monocytes induced T cell proliferation also in the presence of heterologous monocytes (Table II). In the case of LPS-primed monocytes, both autologous and heterologous monocytes effectively activated T cell proliferation, indicating that the LPS-induced T cell proliferation is MHC unrestricted.

Effect of anti-MHC class II antibodies

To further confirm that the LPS-induced T cell proliferation is MHC unrestricted, we investigated whether anti-MHC class II Ab are able to block T cell proliferation. We therefore stimulated PBMC with LPS, PPD, or TT in the presence of anti-HLA-DP, -DQ, and -DR Ab. As shown in Table III, the PPD- or TT-induced T cell proliferation was inhibited by anti-MHC class II Ab, while the LPS-induced T cell proliferation was not affected. Again, these results confirm that LPS induces T cell proliferation by a mechanism that is independent of MHC/T cell receptor interactions.

Monocytes of LPS nonresponders fail to induce proliferation

As shown in our previous reports (12, 13), only PBMC of ~50% of healthy donors show a T cell proliferative response to LPS. Thus, healthy donors can be grouped into LPS responders and nonresponders. T cells of LPS responders showed a good proliferative response to LPS, while T cells of LPS nonresponders failed to respond in the presence of autologous monocytes. Thus, we repeated the experiments

Table I. Effects of anti-CD14 mAb

<table>
<thead>
<tr>
<th>Additors During Priming</th>
<th>DNA Synthesis (cpm/Culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>8932 ± 247</td>
</tr>
<tr>
<td>Anti-CD14 mAb MEM-18 + LPS</td>
<td>564 ± 139</td>
</tr>
<tr>
<td>TT</td>
<td>3921 ± 210</td>
</tr>
<tr>
<td>Anti-CD14 mAb MEM-18 + TT</td>
<td>3942 ± 354</td>
</tr>
<tr>
<td>SEB</td>
<td>7954 ± 467</td>
</tr>
<tr>
<td>Anti-CD14 mAb MEM-18 + SEB</td>
<td>8621 ± 1087</td>
</tr>
<tr>
<td>Medium control</td>
<td>231 ± 43</td>
</tr>
<tr>
<td>Anti-CD14 mAb MEM-18 + medium control</td>
<td>198 ± 23</td>
</tr>
</tbody>
</table>

* Purified monocytes were preincubated with or without anti-CD14 mAb MEM-18 and then primed LPS, TT, or SEB for 1 h. For further details, see Materials and Methods. These primed monocytes (1 x 10^6/ml) were added to purified autologous T lymphocytes (1 x 10^6/ml). DNA synthesis was measured after 4 days in SEB-stimulated cultures or after 7 days in LPS, TT, or unstimulated cultures, respectively. Data are expressed as mean ± SD of three parallel cultures.
expressed as mean ± SD of three independent cultures.

A T lymphocytes (1 × 10^6/ml) were stimulated with LPS (1 μg/ml), TT (10 μg/ml), or SEB (10 ng/ml). For further details, see Materials and Methods. Data are expressed as mean ± SD of three independent cultures.

described above with donors of these two groups. We cocultured primed monocytes of a LPS responder with T cells of a nonresponder and vice versa. Typical results of such experiments are shown in Figure 3. Although T lymphocytes of the LPS nonresponder were unable to proliferate in the presence of LPS-pulsed autologous monocytes, they showed a remarkable proliferative response, when cocultured with LPS-pulsed monocytes from a LPS responder (Fig. 3B). On the other hand, T cells of a LPS responder showed a clear response in the presence of LPS-pulsed autologous monocytes, but only a weak response when cocultured with pulsed monocytes of a LPS nonresponder (Fig. 3A).

The role of costimulatory signals

We previously found that LPS-induced T cell proliferation is strongly dependent on direct cell-to-cell contact between viable monocytes and T lymphocytes. These observations made it likely that interactions of membrane-bound ligand/receptor pairs on monocytes and T lymphocytes were delivering costimulatory signals necessary for activation. CD28 and/or CTLA-4 on T lymphocytes with their ligands CD80 and/or CD86 on monocytes are such ligand/receptor pairs. To investigate the role of these costimulatory molecules during LPS-induced T cell proliferation, blocking experiments were performed with soluble CTLA-4. For this purpose, the chimeric product of CTLA-4 and murine Ig (CTLA-4Ig) was added in different concentrations to LPS-, SEB-, or TT-stimulated cultures. As shown in Figure 4A, LPS-induced T cell proliferation was strongly inhibited by CTLA-4Ig in a dose-dependent manner. With a concentration of 0.3 μg of CTLA-4Ig/ml, only background levels of proliferation were observed. Control experiments showed that the antigenic stimulation of T cells by TT was also inhibited in a dose-dependent manner by CTLA-4Ig but that higher amounts of CTLA-4Ig were required for total inhibition (Fig. 4B). The T cell proliferation induced by the superantigen SEB was not affected by CTLA-4Ig (Fig. 4C).

Induction of CD80 but not of CD86 by LPS

The experiments described above show that interactions of CD28 and/or CTLA-4 on T lymphocytes with their ligands CD80 and/or CD86 on accessory monocytes were necessary for stimulation of human T lymphocytes by LPS. This finding caused us to compare the expression of CD80 and CD86 on monocytes of LPS responders and nonresponders after LPS stimulation. Figure 5 shows the
Experimental results obtained with different donors, i.e., LPS responders as well as LPS nonresponders. As already described by others (21), CD80 is not or only slightly expressed on resting monocytes (Fig. 5). When PBMC were cultured for 2 days in medium alone, only a slight increase of CD80 expression was observed (Fig. 5). In LPS-stimulated cultures, expression of CD80 correlated well with the induction of proliferation of T cells by LPS; CD80 expression was clearly enhanced only on monocytes of LPS responders (Fig. 5A), while there were no or only small changes in CD80 expression on monocytes of LPS nonresponders (Fig. 5B). In contrast to CD80, no reproducible and significant changes of CD86 expression on monocytes induced by LPS were found (data not shown).

Effects of anti-IL-12 and IL-15 mAb

Next, we investigated the role of IL-12 and/or IL-15 in LPS-induced T cell proliferation. Thus, we stimulated PBMC with LPS (1 μg/ml) or PPD (10 μg/ml) in the presence of neutralizing Ab against IL-12, IL-15, or a combination of both. As shown in Table IV, neutralizing anti-IL-12 mAb clearly inhibited LPS-induced T cell proliferation between 50 and 80%. Less marked were the effects of anti-IL-15 mAb; addition of neutralizing anti-IL-15 mAb reduced LPS-induced T cell proliferation by only 20 to 30%. Addition of anti-IL-12 mAb together with anti-IL-15 mAb did not further increase the effects of anti-IL12 mAb alone. In contrast, neutralizing the biologic activities IL-12 or IL-15 by adding blocking mAb had more or less no effect in PPD stimulated cultures.

Discussion

Although stimulation of human T lymphocytes by LPS is by now a known phenomenon, the mechanisms underlying the accessory function(s) of monocytes during LPS-induced T cell proliferation are not well understood. As we have described previously, stimulation of T lymphocytes is strongly dependent on direct cell-to-cell contact between monocytes and T lymphocytes and requires the presence of viable monocytes (12, 13). Furthermore, we have shown that monocytes could not be replaced by B lymphocytes (12) or neutrophils (our unpublished observations). As shown in Figure 1, A and B, accessory monocytes within PBMC can be replaced by LPS-primed monocytes. These primed monocytes were active in stimulating T lymphocytes even in the absence of supplemental LPS. On the other hand, we could show a correlation between the stimulatory capacity of primed monocytes and the amount of surface-bound LPS on these monocytes (Fig. 1, A and C). Only monocytes with saturated LPS-binding sites on their surface were able to induce optimal T cell proliferation. In additional experiments, we investigated the different steps involved in the priming of monocytes by LPS.

In the first step, interaction of LPS with monocytes was strongly dependent on membrane-bound CD14, as preincubation of monocytes against IL-12, IL-15, or a combination of both. As shown in Table IV, neutralizing anti-IL-12 mAb clearly inhibited LPS-induced T cell proliferation between 50 and 80%. Less marked were the effects of anti-IL-15 mAb; addition of neutralizing anti-IL-15 mAb reduced LPS-induced T cell proliferation by only 20 to 30%. Addition of anti-IL-12 mAb together with anti-IL-15 mAb did not further increase the effects of anti-IL12 mAb alone. In contrast, neutralizing the biologic activities IL-12 or IL-15 by adding blocking mAb had more or less no effect in PPD stimulated cultures.
Effects of anti-IL-12 and anti-IL-15 mAb on LPS-induced T-cell proliferation

<table>
<thead>
<tr>
<th>Donor</th>
<th>Stimulus</th>
<th>Added mAb</th>
<th>cpm/Culture</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LPS</td>
<td>None</td>
<td>11,770 ± 990</td>
<td>80 ± 2</td>
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<tr>
<td></td>
<td></td>
<td>Anti-IL-12</td>
<td>2,320 ± 290</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Anti-IL-15</td>
<td>8,500 ± 1,790</td>
<td>28 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-IL-12 + anti-IL-15</td>
<td>2,030 ± 600</td>
<td>83 ± 5</td>
</tr>
<tr>
<td></td>
<td>PPD</td>
<td>None</td>
<td>21,390 ± 2,050</td>
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<td></td>
<td>Anti-IL-12</td>
<td>20,070 ± 1,730</td>
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<tr>
<td></td>
<td></td>
<td>Anti-IL-15</td>
<td>19,600 ± 1,430</td>
<td>9 ± 7</td>
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<td></td>
<td></td>
<td>Anti-IL-12 + anti-IL-15</td>
<td>18,050 ± 760</td>
<td>16 ± 4</td>
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<tr>
<td>2</td>
<td>LPS</td>
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<td>25,160 ± 2,600</td>
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<td>Anti-IL-12</td>
<td>6,630 ± 3,070</td>
<td>74 ± 12</td>
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<td>18,800 ± 3,590</td>
<td>25 ± 14</td>
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<td>4,520 ± 1,360</td>
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<td></td>
<td>PPD</td>
<td>None</td>
<td>47,500 ± 3,480</td>
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<tr>
<td>3</td>
<td>LPS</td>
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<td>2,500 ± 600</td>
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<td></td>
<td>PPD</td>
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<td></td>
<td></td>
<td>Anti-IL-12 + anti-IL-15</td>
<td>7,590 ± 530</td>
<td>None</td>
</tr>
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</table>

* PBMC (1 × 10⁶/ml) were stimulated with LPS (1 μg/ml) or PPD (10 μg/ml) in the presence of anti-IL-12 mAb (10 μg/ml), anti-IL-15 mAb (10 μg/ml), or a combination of both Ab and cultured for 7 days. For further details, see Materials and Methods. Data are expressed as mean ± SD of three independent cultures.

As shown in Figure 2, further steps of LPS/monocyte interactions were sensitive to the lysosomotropic agent ammonium chloride. Ammonia has been shown to alkalize lysosomal intracellular compartments and thus to prevent proteolysis of proteins (27). As already described, stimulation of T cells by protein recall Ag, like TT, requires Ag processing (27), while in case of T cell stimulation by the superantigen SEB Ag processing is not necessary (28). Surprisingly, T cell stimulation by the nonprotein compound LPS was also sensitive to ammonium chloride. Whether this sensitivity to ammonia reflects processing steps of LPS or whether ammonia is simply inhibiting the endosomal traffic and processing of secretory products requires further investigations.

Next, we investigated whether or not interaction of T cells with LPS-primed monocytes is MHC restricted. Thus, we cocultured purified T lymphocytes with primed autologous or heterologous monocytes. As shown in Table II, the interaction of LPS-primed monocytes with T cells was not MHC restricted, since primed autologous monocytes could be replaced effectively by primed heterologous monocytes. These results were confirmed by the finding that Ab against MHC class II had no effect on LPS-induced T cell proliferation (Table III).

Interestingly, only LPS-primed monocytes of LPS responders were able to induce T cell proliferation (Fig. 3). Thus, it is not the T cell repertoire that is the limiting factor for a healthy donor to become a LPS responder, but the capability of the monocytes to provide optimal help in the form of costimulatory signals for LPS-induced T cell proliferation.

Beside MHC/T cell receptor interactions, several ligand/receptor pairs are described that deliver costimulatory signals for optimal T cell proliferation. Interaction of CD28 and/or CTLA-4 on T lymphocytes with their ligands CD80 (B7.1) and CD86 (B7.2) on monocytes deliver such costimulatory signals (29, 30). In blocking experiments, we investigated the role of these costimulatory signals during LPS-induced T cell proliferation by the use of soluble CTLA-4Ig. As shown in Figure 3, T cell proliferation induced by LPS or TT was inhibited by CTLA-4Ig in a dose-dependent manner, while superantigen-induced T cell proliferation was not affected. These experiments indicate that costimulatory signals through CD28 and/or CTLA-4 are required for optimal stimulation of human T lymphocytes by LPS and by the recall Ag TT. On the other hand, superantigenic stimulation of T cells was not dependent on B7 costimulatory interactions (28).

The finding that interactions between CD28/CTLA-4 on T lymphocytes with their ligands CD80/CD86 on monocytes were necessary for optimal T cell stimulation made it reasonable to assume that these ligands were differentially regulated on monocytes of LPS responders and LPS nonresponders. Thus, we compared the expression of CD80 or CD86, respectively, on monocytes of LPS responders and nonresponders before and after LPS stimulation (Fig. 5). Indeed, there was a clear correlation of CD80 expression on monocytes after LPS stimulation and their capacity to support T cell proliferation. The expression of CD80 was only enhanced in the group of LPS responders. On the other hand, there was no significant enhancement of the CD86 expression on monocytes of LPS responders and nonresponders. Thus, it was also demonstrated that healthy donors, with regard to the induction of CD80 expression by LPS, can be separated into two groups: one, in which CD80 expression could not be induced by LPS; and another, in which CD80 was inducible by LPS. These observations therefore correlate well with the data presented here.

Blocking of the biologic activity of IL-12 by neutralizing anti-IL-12 mAb resulted in a clear reduction of T cell proliferation, while blocking with neutralizing anti-IL-15 mAb had only minor effects. Thus, beside B7/CD28 interactions, IL-12 also seems to play a crucial role in LPS-induced T cell proliferation, while IL-15 plays a minor role. IL-12 is a strong immunoregulator (26) that synergizes with B7/CD28 interaction in inducing proliferation of human T lymphocytes (32). However, it was shown that IL-12 alone is not able to induce proliferation in resting T lymphocytes.
but stimulates activated T cells or T cell clones (33). Whether LPS, in our experimental system, induces T cell proliferation of in vivo preactivated T lymphocytes by inducing the expression of CD80 on monocytes and their IL-12 secretion is therefore of main interest in our future studies.

In conclusion, LPS-induced T cell activation seems to be based on thus far unknown mechanisms with MHC-unrestricted accessory cell requirements different from classical antigenic or superantigenic stimulation. Recognition of endotoxin by LPS-primed monocytes depends on CD14, and LPS-primed monocytes provide costimulatory signals through CD28 and/or CTLA-4, CD80 interactions, and presumably IL-12 secretion. Whether the status of LPS responders or nonresponders regarding the induction of CD80 expression and T cell activation is of clinical significance, e.g., during Gram-negative sepsis or endotoxemia, remain to be investigated.

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References


