Regulation of Dendritic Cell Numbers and Maturation by Lipopolysaccharide In Vivo

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Summary

Dendritic cells (DC) are described as “nature’s adjuvant,” since they have the capacity to sensitize T cells in vivo upon first encounter with the antigen. The potent accessory properties of DC appear to develop sequentially. In particular, the ability to process antigens and to sensitize naive T cells develops in sequence, a process termed “maturation” that is well described in vitro. Here, we obtain evidence for maturation in vivo in response to the bacterial product lipopolysaccharide (LPS). Before LPS treatment, many DC are found at the margin between the red and white pulp. These cells lack the M342 and DEC-205 markers, but process soluble proteins effectively. 6 h after LPS, DC with the M342 and DEC-205 markers are found in increased numbers in the T cell areas. These cells have a reduced capacity to process proteins, but show increases in the B7 costimulator and T cell stimulatory capacity. 48 h after LPS, the number of DC in the spleen is reduced markedly. We interpret these findings to mean that LPS can cause DC in the marginal zone to mature and to migrate into and then out of the T cell areas.

Optimal activation of T lymphocytes depends on TCR interactions with peptide/MHC complexes in conjunction with costimulatory signals that are typically delivered by the same APC. The costimulatory signal is antigen nonspecific, and is delivered by CD28 or CTLA4 on T cells that interact with a ligand, B7-1 (CD80) or B7-2 (CD86), that is present on the APC (1). The role of costimulatory molecules has been clearly demonstrated by studies showing that antigenic stimulation of T cells in vitro in the absence of costimulation leads to aborted T cell proliferation and the development of functional unresponsiveness or clonal anergy of T cells (2). Injection of CTLA4Ig, a soluble form of the extracellular domain of CTLA-4 with high avidity for B7-1 and B7-2, suppresses T cell–dependent antibody responses in vivo (3). CTLA4Ig is also capable of blocking in vitro proliferation in assays of allograft rejection (4, 5), as well as preventing xenograft rejection (6) and prolonging allograft rejection (7, 8). These data show that B7/CD28-CTLA4 activation pathways play an important role in regulating in vivo T cell responses.

Among the population of APC, which in the mouse comprises dendritic cells (DC), B lymphocytes, and macrophages, DC appear to have the unique capacity to activate naive T cells (9). In vitro, they are essential to trigger a primary antibody response (10) and to generate antiviral CTL (11, 12), and they strongly stimulate CD4+ (13) and CD8+ (14) cells in the primary mixed leukocyte reaction. In vivo, DC appear to play a major role in initiating various T cell immune responses, such as contact sensitivity (15–17) and allograft rejection (18–21). DC pulsed extracorporeally can efficiently induce cellular (22) and T cell–dependent humoral (23, 24) responses in mice. The property of isolated DC to sensitize quiescent T cells upon the first encounter with the antigen correlates with their capacity to express very high levels of antigen/MHC peptides, as well as B7-1 and B7-2 costimulatory molecules (5, 25, 26).

The first step of the immune response leading to efficient activation of antigen-specific, naive T cells in situ is still poorly understood. DC are likely to be the adjuvant of the immune system in vivo. DC are motile and efficiently cluster T cells, are widely distributed in tissues, carry antigens that are administered intradermally or intravenously, and circulate through lymph or blood probably in route to lymphoid organs (for review see reference 9). The analysis of cryostat sections (26), however, revealed that B7-1 and B7-2 staining was weak or negative in most nonlymphoid organs, but strong in select sites in lymphoid organs. In particular, in the spleen, both MHC class II and B7-2 were expressed strongly on dendritic profiles in the T cell re-
dendritic cells derived from the marginal zone. This migration is independent of T cell stimulation. In our system, we have used a monoclonal antibody directed against a marker of activated and migrating cells (CD11c, also known as CR3). The CD11c+ cells are detectable in the marginal zone of BALB/c mouse spleens and are absent from the periphery. In this study, we have used this CD11c+ cell population as a surrogate for migrating dendritic cells. The localization of CD11c+ cells in the marginal zone suggests that they could be responsible for the migration of dendritic cells from the marginal zone to the T cell area. In this paper, we show that systemic administration of lipopolysaccharide (LPS) induces the migration of most splenic DC from the marginal zone to the T cell areas within 4-6 h. This movement parallels a maturation process, as assessed by downregulation of processing capacity and upregulation of immunostimulatory properties. Unexpectedly, 48 h after LPS injection, the number of splenic DC decreased markedly, an observation that correlates with an impaired capacity to activate naïve T lymphocytes in vitro and in vivo.

In Vivo Treatment

Endotoxin Injection. BALB/c mice were injected intravenously into the lateral tail vein with 25 µg LPS solubilized in pyrogen-free NaCl 0.9%. Control animals were injected with the same volume of NaCl.

T Cell Activation In Vivo. LPS-treated and control BALB/c mice were injected with either 50 µg SEB or 50 µg anti-CD3e solubilized in pyrogen-free NaCl 0.9%. The mice were bled 2 h later, and the sera were assayed for IL-2 content using a standard bioassay using IL-2-dependent, IL-4-insensitive subclone of the CTLL cell line. IFN-γ was quantitated by two-site ELISA using mAbs F1 and Db-1, kindly provided by Dr. Billau (Katholieke Universiteit Leuven, Leuven, Belgium) and P.H. Van Der Meide (TNO Health Research, Rijswijk, The Netherlands), respectively.

Cell Lines

The I-Eα-restricted, myogobin-specific T cell hybridoma 13-26-8-HG.1 (33) was kindly provided by Dr. A. Livingstone (Basel Institute of Immunology, Basel, Switzerland). The I-Eα-restricted, pork insulin-specific T cell hybridoma B8P4.1C3 (34) was kindly provided by Dr T. Delovitch (John P. Robarts Research Institute, London, Ontario, Canada).

Flow Cytometry

Cells were analyzed by flow cytometry with a FACScan® cytometer (Becton Dickinson & Co., Mountain View, CA). The cells were preincubated or not (see figure legends) with 2.4G2 (a rat anti-mouse Fc receptor mAb) for 10 min before staining to prevent antibody binding to FcR, and were incubated with fluorescein-coupled mAb 14.4.4 (murine IgG2a anti-I-Eα, available through American Type Culture Collection, Rockville, MD), N418 (hamster antimurine CD11c; 35), 1610A1 (hamster anti-B7-1; 36), GL1 (rat IgG2a anti-B7-2; 37) with PE-coupled CD45R/B220 (rat IgG2a; Pharmingen, San Diego, CA) or with biotinylated N418 followed by avidin coupled to PE or with biotinylated CD11b/Mac1 (PharMingen) revealed by avidin coupled to fluorescein. Cells were gated according to size and scatter to eliminate dead cells and debris from analysis.

Purification of Low Density Spleen Cells and Fresh DC

Spleens were digested with collagenase (CLSIII; Worthington Biochemical Corp., Freehold, NJ) and separated into low and high density fractions on a BSA gradient (Bovuminar Cohn fraction V powder; Armour Pharmaceutical Co., Tarrytown, NY), according to a procedure described by Crowley et al. (38). A further purification of fresh DC (without in vitro culture) by enrichment on a Mini Macs column was sometimes performed, according to the manufacturer’s recommendations (Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany). In brief, low density spleen cells were incubated with biotinylated N418, washed, and incubated with streptavidin microbeads. Cells were then positively enriched on a Mini Macs column. More than 85% of these cells expressed N418 and MHC class II. For FACScan® analysis, a gate was performed on N418-positive cells.

Figure 1. LPS induces migration of N418-positive cells from the marginal zone of the spleen to the T cell area. Immunoperoxidase labeling of cryostat sections from spleens of control mice (A-D) and mice treated 6 h (E-H) or 48 h (I-L) previously with 25 µg LPS intravenously. Sections were stained with anti-CD11c mAb (A, E, and I) with anti-DEC-205 mAb (B, F, and J), with N414 mAb (C, G, and K), or with anti-CD4 mAb (D, H, and L). The sections stained with anti-CD11c and anti-CD4 were counterstained with hematoxylin; the sections stained with NLDC-145 and M342 were counterstained with methyl green. The original magnification was 10. ▼, white pulp; *, red pulp; →, marginal zone.
**In Vitro Responses**

The complete medium used in all experiments was RPMI 1640 (Seromed Biochem KG, Berlin, Germany) supplemented with 2% HY ultraser (a serum-free medium supplement purchased from GIBCO BRL, Merelbeke, Belgium) or 10% FCS, penicillin, streptomycin, nonessential amino acids, sodium pyruvate, 2-ME, and L-glutamine (Flow ICN Biomedicals, Bucks, UK). Splenic T cells were purified by depletion of adherent cells by passage over Sephadex G10 (Pharmacia Bioprocess, Uppsala, Sweden) and complement mediated lysis with a cocktail of anti-APC antibodies. T cells were stimulated with graded doses of SEB in the presence of γ-irradiated (3,000 rads) spleen cells or fresh DC in round-bottom 96-well plates, or were cultured in the presence of various numbers of γ-irradiated (3,000 rads) allogeneic spleen cells. Cultures were maintained at 37°C in a humidified incubator (7% CO2). The supernatants were collected after 12–48 h of culture, frozen, and assayed for IL-2 content (see above).

**Immunohistochemistry**

Tissues samples were frozen in isopentane and 12-μm cryostat sections were prepared. Samples were fixed in acetone for 10 min, air-dried, and incubated in PBS containing 0.5% of blocking reagent (BR) from Boehringer for 30 min. Sections were incubated with biotinylated anti-CD11c mAb followed by streptavidin-PE and for green fluorescence with anti-I-E, anti-B7-1, or anti-B7-2 mAbs coupled to fluorescein. The DC-enriched population (see Materials and Methods) contained at least 85% CD11c-positive cells. The data represent the expression of MHC class II, B7-1, and B7-2 on cells gated for CD11c expression.
The injection of LPS leads to a redistribution of DC markers in the spleen, suggesting that DC have migrated from the marginal zone to the T cell area. Fig. 1 (middle panel) shows serial sections stained with various antibodies. Most N418-positive (E) cells were detected in association with T cells (H) 6 h after injection of LPS. Adjacent sections of the T cell areas were strongly stained with NLDC-145 (F) and M342 (G) antibodies. 48 h after endotoxin administration (Fig. 1, right panel), the overall number of splenic DC was strongly reduced, since only few cells in the T cell areas (L) stained with N418 (I), NLDC-145 (J), or M342 (K).

Splen ic DC Upregulate MHC Class II and B7 Expression Early after LPS Injection. Since the expression of M342 and NLDC-145 is weak or absent on freshly isolated DC and increased after culture and maturation in vitro (40, 41), we tested whether LPS could induce DC maturation in vivo. LPS was injected intravenously, and 6 h later, low density spleen cells were enriched for N418+ cells (see Materials and Methods), and the expression of MHC class II and costimulatory molecules (B7.1 and B7.2) was analyzed by FACS®. As shown in Fig. 2, DC isolated from LPS-treated mice displayed increased levels of I-E, B7-1, and B7-2 molecules, as compared to control animals, whereas the expression of CD11c remained unchanged.

LPS Induces Functional Maturation of Splenic DC within a Few Hours. One of the hallmarks of maturation of DC upon culture in vitro is an increased capacity to sensitize naive T cells and a reciprocal downregulation of the capacity to process protein antigens (26–30). To test whether the injection of LPS and the subsequent upregulation of MHC class II and costimulatory molecules on DC were associated with a functional maturation in vivo, splenic DC were purified from naive or LPS-injected animals and immediately tested as accessory cells for the presentation of SEB to syngeneic naive T cells. This superantigen does not require processing and is best presented by DC (42, 43). As shown in Fig. 3 A, DC enriched from animals that had been injected with LPS 6 h earlier were better activators of T cells than DC from untreated animals. The increased immunostimulatory properties of DC from LPS-treated mice were inversely correlated with their capacity to process and present a protein antigen, as assessed by a decreased ability to present insulin to T cell hybridoma (Fig. 3 B). The capacity of DC to present antigens that do not need processing (SEB or peptides) to T cell hybridoma remained unchanged 6 h after endotoxin administration (data not shown).

LPS Injection Results in the Loss of N418-positive Cells from Mouse Spleen after 2 d. The staining of spleen cryosections (Fig. 1) revealed that the movement of DC induced early by LPS was followed by a strong reduction of N418+ cells 48 h later. To further document this observation and to analyze whether the loss of N418 staining in cryosections corresponded to the absence of DC or to a selective loss of N418 marker, we performed immunofluorescence studies on DC-enriched, low-density spleen cells. Fig. 4 represents the contour profiles of cells double-stained with B220 in red, and either N418 or 14-4-4 (anti-I-E d) in green. The data show that low density spleen cells from LPS-injected mice lack the DC population identified as N418 (squares) or LPS 6 h earlier (circles) were

![Figure 3.](image_url)

**Figure 3.** LPS induces functional maturation of splenic DC within a few hours. N418-positive cells enriched from low density spleen cells from BALB/c mice injected with NaCl (circles) or LPS 6 h earlier (squares) were cultured with (A) 2 × 10⁶ syngeneic T cells in the presence of serial dilutions of SEB or (B) with 5 × 10⁴ T cell hybridoma B8.P4.1.C3 and 200 μg/ml pork insulin. IL-2 production was quantitated from the 12-h (A) or 24-h (B) culture supernatants.
control  

LPS 2 days  

LPS 15 days  

Figure 4. LPS injection results in the loss of N418-positive cells from mouse spleen after 2 d. Two-color immunofluorescence analysis contour plots. Spleen cells from the low buoyant density fraction were stained for red fluorescence with PE anti-B220 mAb and for green fluorescence with anti-I-E^d or anti-CD11c mAb coupled to FITC. Cells were from a pool of two BALB/c mice and injected 48 h or 15 d previously with NaCl (control) or 25 μg LPS. Boxes on the contour plots define selected populations. DC were identified as B220/CD45R^-, I-E^d^+ or B220/CD45R^-, CD11c^+.

LPS injection. Similar data were obtained when syngeneic T lymphocytes were stimulated by SEB in the presence of irradiated spleen cells from both groups (Fig. 5 B). By contrast, spleen cells from control and endotoxin-injected mice induced similar IL-2 production by CD28-independent T cell hybridoma, which has been shown to respond optimally to T cell receptor ligation in the absence of B7-related costimulatory function (Fig. 5 C). The addition of antibodies specific for CD28 overcame the APC defect of spleen cells from LPS-injected mice in the MLR (Fig. 5 D), suggesting that spleen cells from treated animals retained the ability to generate a TCR ligand, but displayed defective costimulatory function.

In vivo immune response of control and LPS-treated mice after injection of DC-dependent or -independent stimuli. Control and LPS-treated mice were injected with 50 μg SEB or with 25 μg anti-CD3 mAb (mainly presented by FcR^+ B cells or macrophages; 44), and were bled 2 h later to quantify the IL-2 and the IFN-γ produced in the sera. Fig. 6 shows that the injection of the T cell mitogen anti-CD3 mAb resulted in high production of IL-2 and IFN-γ in both groups. By contrast, the secretion of IL-2 and IFN-γ after stimulation by SEB was reduced in mice injected with endotoxin, as compared to untreated animals.

Discussion

The unique capacity of splenic DC to optimally activate naive T cells has been extensively demonstrated in vitro

Table 1. Effect of LPS on Splenic Cell Populations

|            | Control* | LPS  |
|------------|----------|------|
| DC^+       | 0.84^5   | 0.20 |
| B cells    | 39.0     | 49.0 |
| Macrophages| 3.3      | 5.5  |

*The numbers of spleen cells were: 9 × 10^7 (control); 1.4 × 10^8 (LPS-injected 48 h earlier).
^Splenic cell populations were identified as follows; DC, CD11c^+; B lymphocytes, CD45R/B220^+; macrophages, CD45R/B220^+; CD11b/Mac-1^+.
^Percent of splenic cells.
and in vivo. In particular, purified DC, pulsed extra- corporeally with an antigen, induce specific cellular and humoral responses when injected into syngeneic animals (22, 23). The adjuvant properties of these cultured DC may depend on a process of maturation that occurs "spontaneously" in vitro during the purification steps (26–30). Whether a similar maturation occurs in vivo is still unknown, and it is of major interest to define the conditions that are required for the induction of primary responses in situ. The data presented herein strongly suggest that LPS induces splenic DC maturation in situ, as assessed by upregulation of immunostimulatory properties and downmodulation of processing capacity. These functional changes correlate with a rapid migration of DC to T cell areas, and are followed by the loss of most splenic DC 48 h after LPS injection.

The identification of a specific costimulatory signal such as CD28 has focused attention on the functional heterogeneity of APCs. Indeed, optimal activation of naive T cells requires TCR occupancy by antigen/MHC complexes and additional signal(s), such as CD80, CD86, CD40, etc. The expression of CD80 and CD86 varies among the APC populations and seems to correlate with their immunostimulatory properties. DC appear to have some constitutive costimulatory function in several sites in situ, but more typically, this function rapidly develops upon in vitro culture (26). Steinman’s group has defined two populations of splenic DC that differ by their localization and phenotype. DC in the T cell regions express MHC class II and B7-2, and are recognized by M342 and NLDC-145 mAbs. DC in the marginal zone are M342- and resemble freshly isolated DC that become M342+ upon culture. These authors suggest that DC from the marginal zone may give rise to M342+ cells in T cell areas, and this hypothesis is supported by our data showing that DC migrate from the marginal zone to the T cell area and concomitantly mature into M342+, NLDC-145+ potent accessory cells that express increased levels of class II, B7-1, and B7-2 molecules. We interpret the redistribution of the N418 marker in the spleen 6 h after LPS injection as a migration of DC from the marginal zone to T cell areas, and this hypothesis is supported by our data showing that DC migrate from the marginal zone to the T cell area and concomitantly mature into M342+, NLDC-145+ potent accessory cells that express increased levels of class II, B7-1, and B7-2 molecules. We interpret the redistribution of the N418 marker in the spleen 6 h after LPS injection as a migration of DC from the marginal zone to T cell areas, although it could result from the loss of a subset of DC present in peripheral areas in spleens and from the recruitment of a distinct subset in the central white pulp. Our hypothesis, however, is supported by the observation that N418+ cells are gradually detected in regions located from the periphery to the center of the white pulp 1.5–4 h after LPS injection (data not shown). The phenotypic changes observed 6 h after LPS injection correlate with increased stimulatory properties, as...
assessed by enhanced capacity to induce IL-2 secretion by naive T cells in the presence of SEB. By contrast, the capacity of DC to process native protein such as insulin diminishes after LPS injection, as shown by a decreased ability to trigger IL-2 production by an insulin-specific T cell hybridoma. Collectively, these data strongly suggest that immature DC present in the marginal zone mature after migration to T cell area.

It is noteworthy that recent studies (45) have shown that the NLDC-145 mAb recognizes a receptor, termed DEC-205, with a multilectin domain structure that favors efficient capture and presentation of carbohydrate-bearing antigens. The observation that mature DC lose their capacity to process antigens but display increased expression of DEC-205 is intriguing and suggests that DC maintain their capacity to selectively bind carbohydrates. Since these common constituents of microbial cell walls have structures that are distinct from those of carbohydrates of eukaryotic cell surfaces, mature DC may discriminate self from infectious nonself.

The loss of splenic DC 2 d after endotoxin administration probably reflects a physical disappearance of these cells rather than downmodulation of DC-specific markers, as suggested by the loss of several molecules (class II, CD11c, NLDC-145, and M342), the movement of N418* cells early after injection, and the corresponding functional deficiency. In vitro, we showed that the capacity to sensitize naive T cells, a unique property of DC, is impaired in mice that were injected with LPS 2 d earlier. By contrast, spleen cells from treated mice retain the capacity to activate a costimulatory-independent T cell hybridoma, as well as naive T cells in the presence of an anti-CD28 mAb that mimics B7 engagement. In vivo, activation of T cells by DC-dependent T cell mitogen (SEB) is impaired in LPS-treated mice, as assessed by IL-2 and IFN-γ secretion. By contrast, similar levels of both cytokines are released in the sera of mice after the injection of a DC-independent stimulus such as anti-CD3 mAb. These observations strongly suggest that the inability to induce primary responses in vitro and in vivo results from the selective loss of cells from the dendritic lineage, which displays some costimulatory function in vivo in the absence of intentional stimulation.

The disappearance of DC could be caused by cell exhaustion, migration out of the spleen, or death (by induction of apoptosis?). Fas ligand and TNF have indeed been found to induce apoptosis in several cell types (46, 47). A similar loss of DC was induced by LPS in mice displaying defective Fas ligand (our unpublished observations), however, suggesting that the induction of programmed cell death via Fas/FasL is not involved in this phenomenon. Alternatively, mature DC could migrate from the T cell area to the liver, where they would be eliminated, or to the celiac nodes, although there is some evidence that DC do not recirculate from blood to lymph (48). It would be of major interest to analyze whether this sequence of events is regulated by DC/T interaction in the presence of antigen. The injection of LPS and the antigen in mice expressing a TCR transgene will help to analyze whether antigen-specific, MHC-restricted interaction between DC and T lymphocytes would lead to reciprocal regulation of function and, in particular, may prevent a rapid loss of DC.

The loss of DC after endotoxin administration could be related to the marked depression of cell-mediated immune functions, often associated with septic shock. Indeed, several lines of evidence suggest that the depression in the patient’s immune function induced by traumatic injury underlies the development of infection and/or sepsis (49–51). The analysis of DC phenotype and function in these patients would help to evaluate the role of DC in the pathogenesis of sepsis.

Our data extend previous studies showing that LPS affects DC populations. Groeneveld and co-workers showed that the number of interdigitating cells was severely decreased in the mouse 48 h after LPS administration (52). More recently, MacPherson et al. reported that injection of LPS in rats caused an increased release of intestinal DC into lymph (53). Similarly, Austyn’s group showed that injection of LPS induced a loss of DC from the heart, kidneys, and epidermal Langerhans cells (54). By contrast, LPS aerosolized has been shown to recruit DC into the conducting
airways (55). Of note, a recent report indicates that inflammatory cytokines or exogenous bacterial products modulate the cell-surface phenotype and the immunostimulatory function of human cultured DC (56).

Migration patterns of DC in the mouse have been extensively studied. Skin painting mice with picryl chloride resulted in an increase in the number of DC in lymph nodes that initiate primary antigen-specific stimulation in vitro and in vivo (57). Donor-derived DC have been shown to move from the nonlymphoid organs to the recipient's spleen, where they trigger allograft rejection (20). Labeled mature DC transferred intravenously into a syngeneic host localized in the spleen and the homing was T dependent (58). After subcutaneous footpad inoculation, DC accumulated in the popliteal nodes (48). These data show that DC migrate from nonlymphoid tissues to T cell areas of lymphoid organs, and our results suggest that this flux may correlate with a functional maturation.

A large array of cytokines and/or inflammatory mediators (including TNF-α, IL-1β, IL-6, IL-12, and GM-CSF) is secreted in mice after LPS injection. We have tested the effect of two cytokines that have been shown to affect DC viability and/or maturation in vitro or in vivo (59-61). The injection of recombinant TNF-α induced migration of DC to the T cell area, followed by loss of these cells, whereas GM-CSF did not affect the DC population (De Smedt, T., and M. Moser, manuscript in preparation). Simultaneous injection of neutralizing anti-TNF-α mAb and LPS, however, did not prevent DC movement, suggesting that additional factors induced by LPS may promote DC migration in vivo. Experiments are in progress to test whether IL-1, which has been shown to induce changes in DC function in vitro (62) and promote DC migration in vivo (59), is involved in this phenomenon.

A direct effect of endotoxin on DC cannot be excluded, however, since treatment of DC by LPS has been shown to lead to increased expression of IL-1, IL-6, and IL-12 mRNA by these cells (63), as well as to upregulate gene expression and induce release of TNF-α in DC clones (64).

The cell population(s) involved in this phenomenon remain(s) to be identified. The injection of LPS in SCID mice results in similar changes in DC (data not shown), suggesting that functional T and B lymphocytes are not required for this phenomenon.

LPS endotoxin from Gram-negative bacteria possesses both harmful and beneficial effects. LPS triggers a host immune response resulting in activation of various cell types and production of multiple cytokines that mediate resistance to the bacteria. An exaggerated inflammatory response to bacterial products, however, may cause irreversible tissue damage leading to death when homeostasis is disrupted. Therefore, it is tempting to speculate that the inhibition of DC function in endotoxemia may constitute a feedback aimed at maintaining the homeostasis of the immune system by inhibiting the function of the most efficient APC in vivo. Such a mechanism would be beneficial to the host by preventing harmful effects of an exaggerated inflammatory response, as well as to the bacteria, by limiting the pathogen-specific immune response. The selective loss of DC after endotoxin administration may provide some insights in bacterial-host relations, and in particular, suggest that bacteria may derive some evolutionary advantage to retain a wall component that is strongly immunogenic, since high doses of LPS may reduce host resistance to the spread of bacteria.

In conclusion, our data support the hypothesis that a critical signal for the initiation of immune responses is provided by microbial infection, as proposed by Janeway (65). LPS induces costimulatory activity for CD4+ T cells on B lymphocytes and macrophages (66), and triggers maturation and migration of DC in vivo (this paper). The upregulation of DC function by microbial products and their subsequent movement to T cell areas result in colocalization of T lymphocytes and mature DC that have encountered microbial antigens. Since immature DC have been shown to take up and process antigen, a property that can be lost in mature DC, it is tempting to speculate that most DC in T cell areas would present infectious non-self-antigens. The positive and negative regulation of antigen presentation by microbial agents may help to gain an understanding of how the immune system efficiently eliminates pathogens while avoiding self-reactivity and controls potentially harmful inflammatory responses.
References

1. Thompson, C.B. 1995. Distinct roles for the costimulatory ligands B7-1 and B7-2 in T helper cell differentiation? Cell. 81:979–982.

2. Schwartz, R.H. 1990. A cell culture model for T lymphocyte clonal anergy. Science (Wash. DC). 248:1349–1356.

3. Linse, S.P., P.M. Wallace, J. Johnson, M.G. Gibson, J.L. Greene, J.A. Leder, B. Singh, and M.A. Tepper. 1992. Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. Science (Wash. DC). 257:792–795.

4. Linse, S.P., W. Brady, M. Ureno, L.S. Grosmaire, N.K. Damle, and J.A. Leder. 1991. CTLA-4 is a second receptor for the B cell activation antigen B7. J. Exp. Med. 174:561–564.

5. Lenschow, D.J., G. Hue-Ting Su, I.A. Zuckerman, N. Nakavi, C.L. Jellis, G.S. Gray, J. Miller, and J.A. Bluestone. 1993. Expression and functional significance of an additional ligand for CTLA-4. Proc. Natl. Acad. Sci. USA. 90:11054–11058.

6. Lenschow, D.J., Y. Zeng, J.R. Thistlethwaite, A. Montag, W. Brady, M.G. Gibson, P.S. Linsey, and J.A. Bluestone. 1992. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. Science (Wash. DC). 257:789–792.

7. Turka, L.A., P.S. Linsey, H. Lin, W. Brady, J.M. Leiden, R. Wei, M.L. Gibson, X. Zhen, S. Myrdal, D. Gordon et al. 1992. T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection in vivo. Proc. Natl. Acad. Sci. USA. 89:11102–11105.

8. Lin, H., S.F. Bolling, P.S. Linsley, R.-Q. Wei, D. Gordon, C.B. Thompson, and I.A. Turka. 1993. Long-term acceptance of high histocompatibility complex mismatched cardiac allografts induced by CTLA-4-Ig plus donor-specific transfusion. J. Exp. Med. 178:1801–1806.

9. Steinman, R.M. 1991. The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. 9:271–296.

10. Inaba, K., M.D. Witmer, and R.M. Steinman. 1984. Clustering of dendritic cells, helper T lymphocytes, and histocompatible B cells, during primary antibody responses in vitro. J. Exp. Med. 160:858–876.

11. Kast, W.M., C.J.P. Boog, P.O. Roes, A.C. Voordouw, and C.J.M. Melief. 1988. Failure or success in the restoration of virus-specific cytotoxic T lymphocytes response defects of dendritic cells. J. Immunol. 143:3188–3193.

12. Macatonia, S.E., P.M. Taylor, S.D. Knight, and B.A. Askonas. 1989. Primary stimulation by dendritic cells induces anti-viral proliferative and cytotoxic T cell responses in vitro. J. Exp. Med. 169:1255–1264.

13. Steinman, R.M., B. Gutchinson, M.D. Witmer, and M.C. Nussenzweig. 1983. Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. J. Exp. Med. 157:613–627.

14. Inaba, K., J.W. Young, and R.M. Steinman. 1987. Direct activation of CD8+ cytotoxic T lymphocytes by dendritic cells. J. Exp. Med. 166:182–194.

15. Britz, J.S., P.W. Askenase, W. Ptak, R.M. Steinman, and R.K. Gershon. 1992. Specialized antigen-presenting cells: splenic dendritic cells and peritoneal exudate cells induced by mycobacteria, activate effector T cells that are resistant to suppression. J. Exp. Med. 155:1344–1356.

16. Macatonia, S.E., S.C. Knight, A.J. Edwards, S. Griffiths, and P. Fryer. 1987. Localization of antigen on lymph nodes dendritic after exposure to the contact sensitizer fluorescein isothiocyanate. J. Exp. Med. 166:1654–1667.

17. Inaba, K., and S.I. Katz. 1990. Phenotypic and functional characteristics of in vivo-activated Langerhans cells. J. Immunol. 145:2791–2796.

18. Lechler, R.I., and J.R. Batchelor. 1982. Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. J. Exp. Med. 155:31–41.

19. Faustman, D., R.M. Steinman, H. Gebel, V. Hauftield, J. Davie, and P. Lacy. 1984. Prevention of rejection of murine islets allograft by pretreatment with anti-dendritic cell antibody. Proc. Natl. Acad. Sci. USA. 81:3864–3868.

20. Larsen, C.P., P.J. Morris, and J.M. Austyn. 1990. Migration of dendritic leukocytes from cardiac allografts into host spleens. A novel pathway for initiation of rejection. J. Exp. Med. 171:307–314.

21. Boog, C.J.P., W.M. Kast, H. Thy, M. Timmers, J. Boes, L.P. de Waal, and C.J.M. Melief. 1985. Abolition of specific immune response defect by immunization with dendritic cells. Nature (Lond.). 318:59–62.

22. Inaba, K., M.T. Metlay, M.T. Crowley, and R.M. Steinman. 1990. Dendritic cells pulsed with antigens in vitro can prime antigen-specific, major histocompatibility complex-restricted T cells in situ. J. Exp. Med. 172:631–640.

23. Sornasse, T., V. Flamand, G. De Becker, H. Bazin, F. Tielemans, K. Thielemans, J. Urbain, O. Leo, and M. Moser. 1992. Antigen-pulsed dendritic cells can efficiently induce an antibody response in vivo. J. Exp. Med. 175:15–21.

24. Flamand, V., T. Sornasse, K. Thielemans, C. Demanet, M. Bakkus, H. Bazin, F. Tielemans, O. Leo, J. Urbain, and M. Moser. 1994. Murine dendritic cells pulsed in vitro with tumor antigen induce tumor resistance in vivo. Eur. J. Immunol. 24:605–610.

25. Larsen, C.P., S.C. Ritchie, T.C. Pearson, P.S. Linsley, and R.P. Lowry. 1992. Functional expression of the costimulatory molecule, B7/B71, on murine dendritic cell populations. J. Exp. Med. 176:1215–1220.

26. Inaba, K., M. Witmer-Pack, M. Inaba, K.S. Hathcock, H. Sakuta, M. Azuma, H. Yagita, K. Okumura, P.S. Linsley, K. Ikehara et al. 1994. The tissue distribution of the B7-2 co-stimulator in mice: abundant expression on dendritic cells in situ and during maturation in vitro. J. Exp. Med. 180:1849–1860.

27. Moser, M., T. De Smedt, T. Sornasse, F. Tielemans, A.A. Chentoufi, E. Muraille, C.G. Fathman, K. Inaba, and R.M. Steinman. 1995. Glucocorticoids down-regulate dendritic cell function in vitro and in vivo. Eur. J. Immunol. 25:2818–2824.

28. Romani, N., S. Koide, M. Crowley, M. Witmer-Pack, A.M. Livingstone, C.G. Fathman, K. Inaba, and R.M. Steinman. 1989. Presentation of exogenous protein antigens by dendritic cells to T cell clones. Intact protein is presented best by immature, epidermal Langerhans cells. J. Exp. Med. 169:1169–1178.

29. Crowley, M.T., K. Inaba, M.D. Witmer-Pack, S. Grezelter, and R.M. Steinman. 1990. Use of the fluorescence activated cell sorter to enrich dendritic cells from mouse spleen. J. Immunol. Methods. 133:55–66.

30. Koch, F., B. Trockenbacher, E. Kämpe, G. Schuler, and N. Romani. 1995. Antigen processing in populations of mature murine dendritic cells is caused by subsets of incompletely matured cells. J. Immunol. 155:93–100.

31. Harding, F.A., J.G. McArthur, J.A. Gross, D.H. Rautel, and
32. Leo, O., M. Foo, D. Sachs, L.E. Samelson, and J. Bluestone. 1987. Identification of a monoclonal antibody specific from a murine T3 peptide. Proc. Natl. Acad. Sci. USA. 84:1374–1378.

33. Razi-Wolf, Z., F. Galvin, G. Gray, and H. Reiser. 1993. Engagement of LFA-1 with ICAM-1 mediates co-stimulatory signal required for cell proliferation of murine T cells. J. Exp. Med. 175:157–162.

34. Naquet, P., J. Ellis, B. Singh, R.S. Hodges, and T.L. Delovitch. 1987. Processing of insulin. I. Analysis of immunogenic peptides and processing requirements for insulin A loop-specific T cells. J. Exp. Med. 163:139-3955–3963.

35. Metlay, J.P., M.D. Witmer-Pack, R. Agger, M.T. Crowley, D. Lawless, and R.M. Steinman. 1990. The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. J. Exp. Med. 171:1753–1771.

36. Rashid, A., M. Galvin, G. Gray, and H. Reiser. 1993. Evidence for an additional ligand, distinct from B7, for CTLA-4 receptor. Proc. Natl. Acad. Sci. USA. 90:11182–11186.

37. Hathcock, K.S., G. Lazio, H.B. Dickler, J. Bradshaw, P. Lindsey, and R.J. Hodes. 1993. Identification of an alternative CTLA-4 ligand costimulatory for T cell activation. Science (Wash. DC). 262:905–907.

38. Crowley, M., K. Inaba, M. Witmer-Pack, and R.M. Steinman. 1989. The cell surface of mouse DC: FACS analyses of DC from different tissues including thymus. Cell. Immunol. 118:108–125.

39. Kraal, G., M. Breel, M. Jnase, and G. Bruin. 1986. Langerhans cells, veiled cells, and interdigitating cells in the mouse recognized by a monoclonal antibody. J. Exp. Med. 163:981–997.

40. Agger, R., M. Witmer-Pack, N. Romani, H. Stossel, W.J. Swiggard, J.P. Metlay, E. Storozynsky, P. Freimuth, and R.M. Steinman. 1992. Two populations of splenic dendritic cells detected with M342, a new monoclonal to an intracellular antigen of interdigitating dendritic cells and some B lymphocytes. J. Leukoc. Biol. 52:34–42.

41. Inaba, K., W.J. Swiggard, M. Inaba, J. Melzter, A. Mirza, T. Sasagawa, M.C. Nussenzweig, and R.M. Steinman. 1995. Tissue distribution of the DEC-205 protein that is detected by the monoclonal antibody NLDC-145. 1. Expression on dendritic cells and other subsets of mouse leukocytes. Cell. Immunol. 163:148–156.

42. Bhardwaj, N., J.W. Young, A.J. Nisanian, J. Baggers, and R.M. Steinman. 1993. Small amounts of superantigens, when presented on dendritic cells, are sufficient to initiate T cell responses. J. Exp. Med. 178:633–642.

43. Muraille, E., G. De Becker, M. Bakkus, K. Thieleman, J. Urbain, M. Moser, and O. Leo. 1995. Co-stimulation lowers the threshold for activation of naive T cells by bacterial superantigens. Int. Immunol. 152:5028–5219.

44. Leo, O., D.H. Sachs, L.E. Samelson, M. Foo, R. Quinones, R. Gress, and J.A. Bluestone. 1986. Identification of monoclonal antibodies specific for the T cell receptor complex by Fc-receptor-mediated CTL lysis. J. Immunol. 137:3874–3880.

45. Jiang, W., W.J. Swiggard, C. Heufler, M. Peng, A. Mirza, R.M. Steinman, and M.C. Nussenzweig. 1995. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. Nature (Lond.). 375:151–155.

46. Nagata, S., and P. Golstein. 1995. The Fas death factor. Science (Wash. DC). 267:1449–1456.

47. Vandenabeele, P., W. Declercq, R. Beyaert, and W. Fiers. 1995. Two tumor necrosis factor receptors: structure and function. Trends Cell Biol. 5:392–399.

48. Kupiec-Weglinski, J.W., J.M. Austyn, and P.J. Morris. 1988. Migration patterns of dendritic cells in the mouse. Traffic from the blood, and T cell-dependent and -independent entry to lymphoid tissues. J. Exp. Med. 167:632–645.

49. Abraham, E., and R.F. Regan. 1985. The effects of hemorrhage and trauma on interleukin 2 production. Arch. Surg. 120:1341–1344.

50. Gribb, J.T., J.A. Mannick, D.B. Gough, and M.L. Rodrick. 1991. The role of prostaglandin E2 in immune suppression following injury. Ann. Surg. 214:253–263.

51. Ayala, A., Z.K. Deol, D.L. Lehman, C.D. Herdon, and I.H. Chaudry. 1994. Polymicrobial but not low-dose endotoxin infusion causes decreased splenocyte IL-2/IFN-γ release while increasing IL-4/IL-10 production. J. Surg. Res. 56:579–585.

52. Groeneveld, P.H.P., T. Erich, and G. Kraal. 1986. The differential effects of bacterial lipopolysaccharide (LPS) on splenic non-lymphoid cells demonstrated by monoclonal antibodies. Immunology. 58:285–290.

53. MacPherson, G.G., C.D. Jenkins, M.J. Stein, and C. Edwards. 1995. Endotoxin-mediated dendritic cell release from the intestine. Characterization of released dendritic cells and TNF dependence. J. Immunol. 154:1317–1322.

54. Roake, J.A., A.S. Rao, P.J. Morris, C.P. Larsen, D.F. Hankins, and J.M. Austyn. 1995. Dendritic cell loss from nonlymphoid tissues after systemic administration of lipopolysaccharide, tumor necrosis factor, and interleukin 1. J. Exp. Med. 181:2237–2247.

55. Schon-Hegrad, M.A., J. Oliver, P.G. McMenamin, and P.G. Chaudry. 1995. Endotoxin-mediated dendritic cell release from the intestine. J. Immunol. 154:1317–1322.

56. Austyn, J.M., J.W. Kupiec-Weglinski, D.F. Hankins, and P.J. Morris. 1988. Migration patterns of dendritic cells in the mouse. Homing to T cell-dependent areas of spleen, and binding within marginal zone. J. Exp. Med. 167:646–651.

57. Schon-Hegrad, M.A., J. Oliver, P.G. McMenamin, and P.G. Chaudry. 1995. Endotoxin-mediated dendritic cell release from the intestine. J. Immunol. 154:1317–1322.

58. Austyn, J.M., J.W. Kupiec-Weglinski, D.F. Hankins, and P.J. Morris. 1988. Migration patterns of dendritic cells in the mouse. Homing to T cell-dependent areas of spleen, and binding within marginal zone. J. Exp. Med. 167:646–651.

59. Heufler, C., F. Koch, and G. Schuler. 1988. Granulocyte/macrophage colonystimulating factor and interleukin 1 mediate the maturation of epidermal Langerhans cells into potent immunostimulatory dendritic cells. J. Exp. Med. 167:700–705.

60. Cumberbatch, M., and I. Kimber. 1992. Dermal tumor necrosis factor-α induces dendritic cell migration to draining lymph nodes, and possibly provides one stimulus for Langerhans cell migration. Immunology. 75:257–263.

61. Cumberbatch, M., and I. Kimber. 1992. Tumor necrosis factor-α is required for accumulation of dendritic cells in draining lymph nodes and for optimal contact sensitization. Immu-
62. Koide, S.L., K. Inaba, and R.M. Steinman. 1987. Interleukin 1 enhances T cell-dependent immune responses by amplifying the function of dendritic cells. *J. Exp. Med.* 165:515-530.

63. Kanangat, S., S. Nair, J.S. Babu, and B.T. Rouse. 1995. Expression of cytokine mRNA in murine splenic dendritic cells and better induction of T cell-derived cytokines by dendritic cells than by macrophages during in vitro costimulation assay using specific antigens. *J. Leukoc. Biol.* 57:310-316.

64. Granucci, F., G. Girolomoni, M.B. Lutz, M. Foti, G. Marconi, P. Gnocchi, L. Nolli, and P. Ricciardi-Castagnoli. 1994. Modulation of cytokine expression in mouse dendritic cell clones. *Eur. J. Immunol.* 24:2522-2526.

65. Janeway, C. 1989. Immunogenicity signals 1, 2, 3 ... and 0. *Immunol. Today.* 10:286-289.

66. Liu, Y., and C.A. Janeway. 1991. Microbial induction of costimulatory activity for CD4 T-cell growth. *Int. Immunol.* 3:323-332.