Dendritic Cell Loss from Nonlymphoid Tissues after Systemic Administration of Lipopolysaccharide, Tumor Necrosis Factor, and Interleukin 1

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Summary

Dendritic cells (DC) in nonlymphoid organs can internalize and process foreign antigens before migrating to secondary lymphoid tissues to initiate primary immune responses. However, there is little information on which stimuli promote migration of DC from the tissues. Systemic administration of lipopolysaccharide (LPS), which induces in vivo production of cytokines, led to a reduction in the numbers of major histocompatibility complex class II–positive (Ia^+) leukocytes in mouse hearts and kidneys: >95% of DC were depleted 1–3 d after injection of 50 μg LPS. Several lines of evidence indicated that this response was due to migration of DC rather than loss of Ia expression or cytotoxic effects. In skin of treated mice, the number of Ia^+ epidermal Langerhans' cells (LC) was reduced, and "cords" of Ia^+ leukocytes became evident in the dermis. The latter cells expressed little NLDC145 and may have originated from recruited or resident DC progenitors. Systemic administration of recombinant tumor necrosis factor (rhTNF)-α resulted in a decrease in numbers of Ia^+ cells in heart and kidney and of epidermal LC, and it also induced dermal cords. Administration of a rh-interleukin (IL)-1 resulted in a decrease in Ia^+ cells only in renal medulla, appeared to activate a subset of epidermal LC, and induced dermal cords. Similar microgram doses of rhIL-2 had no obvious effect. Treatment with a neutralizing anti-TNF antiserum before LPS administration inhibited the depletion of LC from skin but not from heart or kidney. Therefore, TNF-α and IL-1α may promote DC migration from nonlymphoid tissues and may have differential effects on different DC populations, but it is unclear whether they act on DC directly or indirectly (e.g., via other cytokines).

Dendritic cells (DC)[1] originate from MHC class II (Ia)–negative progenitors in the bone marrow (1). Within nonlymphoid tissues, they apparently develop into Ia^+ cells with the capacity to take up and process antigens (2). These cells can then migrate into secondary lymphoid tissues and mature into lymphoid DC with the specialized costimulatory functions necessary for T cell activation (3). In previous studies, it was shown that DC can migrate via the blood into the spleen, in addition to their documented migratory route via the lymphatics into lymph nodes (4). Other studies on the behavior of Langerhans' cells (LC) in skin transplants and explants revealed that the cells migrated from the epidermis into the dermis before leaving the tissue and suggested that their migration was promoted by locally produced inflammatory cytokines (5).

The development and maturation of DC are dependent on cytokines such as GM-CSF, TNF-α, and IL-1α in vitro (6), but much remains to be discovered about the mediators of DC migration in vivo. The present report focuses on the response of interstitial DC from mouse heart and kidney (7) and of LC to systemically administered LPS, which has well-documented proinflammatory and adjuvant properties, and to systemic recombinant TNF-α and IL-1α.

Materials and Methods

Experimental Animals

Male C57BL/6 (H-2^b) and BALB/c (H-2^d) mice were obtained from Olac Ltd. (Bicester, UK). C3H/HeN (H-2^k, lps^d) and

[1] *Abbreviations used in this paper:* DC, dendritic cell; FBS, fetal bovine serum; Ia, MHC class II; LC, Langerhans' cell; PD, PBS lacking calcium and magnesium; rh, recombinant human; rm, recombinant murine.

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C3H/HeJ (H-2^k, lps^n) mice were obtained from Jackson Laboratories (Bar Harbor, ME). All animals used were between 6 and 24 wk of age.

Reagents

R10. Complete culture medium (R10) was RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) (both from Gibco BRL, Paisley, UK), 2 mM L-glutamine (BDH Chemicals, Merck Ltd., Atherstone, UK), 25 μM 2-ME (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK), 45 μg/ml penicillin, 45 μg/ml streptomycin, and 90 μg/ml kanamycin (antibiotics from Gibco BRL). PD is PBS lacking calcium and magnesium.

mAbs. The following rat anti-mouse mAbs were used as tissue culture supernatants (TIB and HB refer to American Type Culture Collection [Rockville, MD] designations): M1/9.3.4 (TIB122) anti-CD45; B21-2 (TIB229) anti-$IA^{b,d}$; 2.4G2 (HB 197) anti-CDw32; Ser4 antisialoadhesin (8); F4/80 (HB198) antimacrophage and DC subset; M1/70 (TIB128) anti-CD11b; GK1.5 (TIB207) anti-CD4; 53-6.72 (TIB105) anti-CD8; 2D2C anti-CD44 (9); NLDC145 anti-DC subset (10); and 7/4 antineutrophil and activated macrophage (11). The hamster mAb N418 (HB224) anti-CD11c was generously provided by Dr. R. Steinman (The Rockefeller University, New York). Biotinylated mouse anti-IA^k was obtained from PharMingen (San Diego, CA).

LPS and Recombinant Cytokines

LPS. S-chemotype LPS (L-2637; Sigma Chemical Co., Poole, UK) extracted by the Westphal phenol method and purified chromatography (<1% protein) was dissolved in pyrogen-free normal saline at 200 μg/ml, filtered, and stored at −20°C.

Recombinant Human IL-1α (rhIL-1α). rhIL-1α was generously provided by Dr. P. Lomedico (Hoffmann-La Roche, Inc., Nutley, NJ). The LPS content was reported to be <0.4 endotoxin units/mg protein, and the specific activity was reported to be 3×10^8 U/mg in the D10 assay. It was stored at −70°C.

rhIL-2. rhIL-2 (Cetus Corp., Emeryville, CA) was generously provided by Dr. M. Dallman (Imperial College, London, UK). The LPS content was reported to be <0.006 ng/mg protein by the limulus test, and specific activity was reported to be 1.8×10^7 U/mg. It was stored at −70°C.

rhTNF-α. rhTNF-α (BASF/Knoll, Ludwigshaven, Germany) was generously provided by Dr. R. Evans (Nuffield Department of Anaesthetics, John Radcliffe Hospital, Oxford, UK). The LPS content was reported to be <0.137 ng/mg protein by the limulus test, and specific activity in the L929 cytotoxicity assay was determined to be 2.4×10^7 U/mg. It was stored at −70°C.

Polyclonal Anti-TNF and Control Antisera. A polyclonal antiserum, raised in rabbits against recombinant murine TNF-α (rmTNF-α) and purified by ammonium sulphate precipitation, was generously provided by Dr. E. Havell (Trudeau Institute, Saranac Lake, NY). It had specificity both for TNF-α and TNF-β, and the TNF-neutralizing capacity was determined to be 2.22×10^4 neutralizing unit/mg protein (see below). A control rabbit IgG fraction was similarly prepared from the serum of a New Zealand White rabbit that had been screened for the absence of natural anti-TNF activity; the purified IgG had no detectable TNF-neutralizing activity (see Results).

Administration of LPS and Cytokines. Mice were injected intravenously, intraperitoneally, or subcutaneously with sublethal doses of LPS, recombinant cytokines, polyclonal anti-TNF, or appropriate carrier solutions. At various times after injection, the animals were killed and cryostat sections of organs and tissues were labeled for various markers by immunocytochemical or immunofluorescent techniques.

TNF and Neutralizing Anti-TNF Activity. A modification of a published in vitro assay (12) was used to quantify TNF cytotoxic activity in mouse serum. Briefly, 2×10^4 murine L929B cells in 100 μl R10 were seeded into 96-well flat-bottomed microdilution plates (Falcon 3072; Becton Dickinson, Oxford, UK) and incubated overnight at 37°C in a humidified 5% CO_2 atmosphere. Sequential twofold dilutions of test samples were prepared in R10 containing 2 μg/ml actinomycin D (Calbiochem Corporation, La Jolla, CA) and 100 μl of each dilution was added to replicate wells of the L929B-seeded microdilution plates. After 24 h incubation, cytotoxicity was scored microscopically and by crystal violet absorbance. The cytotoxicity titer (U/ml) was defined as the highest dilution of test sample that caused the destruction of at least 50% of the L929B cells. To ensure specificity of the assay, additional aliquots of each sample were preincubated for 1 h with an excess of the polyclonal anti-TNF antiserum (above) and tested in parallel with test samples.

To assay the neutralizing capacity of the anti-TNF antiserum, sequential twofold dilutions of test samples in R10 containing 2 μg/ml actinomycin D were incubated with an rhTNF-α standard (National Institute of Biological Standards, Potters Bar, UK) at 10 U/ml for 1 h at 37°C. The samples were added to replicate wells of L929B-seeded microdilution plates, and the cytotoxicity titer was determined as above. One neutralizing unit was defined as the concentration of antiserum required to neutralize 10 U/ml of rhTNF-α in vitro.

Tissue Samples

Cryosections. Tissue samples were embedded in OCT compound (Tissue-Tek®; Miles, Inc., Elkhart, IN), and 7–10-μm cryosections were prepared and stored at −30°C.

Skin Explants. Ear skin was split into dorsal and ventral portions, and epidermal and dermal sheets were prepared before (fresh skin) or after culture on R10 for 1–3 d (skin explants) as described (5).

Immunocytochemistry of Cryosections. Samples were fixed in acetone (BDH Chemicals, Merck Ltd.) for 10 min, air dried, and rehydrated. Endogenous peroxidase activity was blocked by incubating with a solution of 0.3% H_2O_2 and 0.1% NaN_3 in PD containing 1% FBS for 10 min at room temperature. Cryosections were incubated with saturating concentrations of rat mAb supernatant followed by peroxidase-conjugated mouse anti-rat IgG (1:50; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). To label C3H/HeN and C3H/HeJ cryosections for IA^k, biotinylated mouse anti-IA^k (Pharmingen) was followed by Extravidin-peroxidase (1:50; E-2886; Sigma Chemical Co.). Incubations were for 45 min at room temperature, and all reagents and washes (three times) between each stage were in PD containing 1% FBS. The peroxidase was developed with a solution containing 10 mg 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical Co.) in 20 ml PD and 160 μl 3% H_2O_2. Slides were counterstained with hematoxylin (Gil's No. 2; Sigma Chemical Co.) or 1% eosin (E-4382; Sigma Chemical Co.) and mounted with DPX (BDH Chemicals, Merck Ltd.).

Immunofluorescent Labeling of Epidermal and Dermal Sheets. For single-color labeling, epidermal and dermal sheets were fixed in acetone for 20 min, air dried, and rehydrated. They were then incubated with saturating concentrations of rat mAb (TIB229) su-

pernatant followed by FITC mouse anti-rat IgG (1:50; Jackson ImmunoResearch Laboratories, Inc.). For two-color labeling, tissue sheets were fixed and rehydrated, as above, and incubated sequentially with saturating concentrations of primary rat mAb, FITC mouse anti-rat IgG (1:50; Jackson ImmunoResearch Laboratories, Inc.), FITC-conjugated Fc-specific goat anti-mouse IgG (1:50; to amplify the FITC signal; Sigma Chemical Co.), normal rat serum (1:50; to reduce backgrounds), saturating concentrations of biotin-TIB122 or biotin-TIB229 (for $CD45^+$ or Ia^+ cells, respectively), and Texas red streptavidin (1:1,000; Jackson ImmunoResearch Laboratories, Inc.).

Incubations were for 90 min at room temperature (20 min for rat serum), and all reagents and washes (three times) between each stage were in PD containing 1% FBS. Slides were mounted with Aquamount (BDH Chemicals, Merck Ltd.).

Analysis of Labeled Cryostat Sections. The number of DC and other leukocytes in hearts and kidney cortex was quantified by direct counts of cells per unit area on immunoperoxidase-labeled cyrostat sections. The counts were made blinded as to the treatment received, and between 5 and 15 randomly selected fields were counted for each mAb on sections from each individual animal. Results were expressed as the mean (± SD) cells per field or per square millimeter.

In the kidney medulla, where the densely packed leukocytes were difficult to distinguish from one another, direct counts were unreliable and a morphometric method was used (13). The method was also applied to the kidney cortex. Briefly, a grid (or graticule) was superimposed on the tissue section and the number of grid intersections overlying positively labeled cells was counted. The proportion of the area labeled was the ratio of the number of positive intersections to the total number of grid intersections. Counts were performed by a single observer, blinded as to the treatment received, and results were reported as the percent area positive. For each of two labeled sections per kidney, three randomly selected fields (at a magnification of 250) were counted. Kidney cortex and medulla were analyzed separately. To maintain an SE of <10%, a 441-point graticule was used.

Analysis of Epidermal Sheets. The LC population density within epidermal sheets was determined by direct counts of labeled cells within a defined area of an ocular grid at a magnification of 625. Generally, 10 randomly selected fields on sheets from each animal were counted, and results were expressed as the mean (± SD) cells per field or per square millimeter. The distribution of LC within the sheets was remarkably uniform, and, hence, relatively small changes in cell density could be detected reliably.

Cardiac Transplantation. Hearts from C57BL/6 (Ia^b) donors were transplanted as fully vascularized heterotopic grafts into C3H/HeN (Ia^k) recipients as described (5). Recipients received 50 μg LPS s.c. 6 h after transplantation. They were killed after a further 24 h, and their spleens were examined for migratory donor DC (14).

Migratory donor cells within the recipient spleen were identified by labeling 7 μm cryosections with mAb specific for donor Ia. The cryosections were fixed in acetone (BDH Chemicals, Merck Ltd.) for 10 min, air dried, rehydrated, and incubated (45 min, room temperature) with saturating concentrations of rat mAb (TIB229; anti-$Ia^{b,d}$) supernatant followed by FITC-mouse anti-rat IgG (1:50; Jackson ImmunoResearch Laboratories, Inc.). To estimate the density of migratory donor cells within the recipient spleens, the number of donor Ia^+ cells in 100–200 fields (at a magnification of 312) was counted, and the density was expressed as the mean number of cells per square millimeter.

Statistics. Statistical significance was determined by applying the Student's *t* test, but, when the data was clearly not normally distributed, nonparametric statistics (e.g., Mann-Whitney test) were applied.

Results

LPS Depletes Ia^+ Cells from Mouse Heart and Kidney

LPS was administered to mice intravenously or subcutaneously, and the number of Ia^+ cells in cryosections of hearts and kidneys was quantified. At 48 h after a single injection of 50 or 100 μg LPS, the number of Ia^+ cells in both tissues was profoundly reduced compared to tissues of untreated or carrier-injected mice (Fig. 1). This was preceded, at 4 h, by a marked increase in the size of interstitial cells in the renal medulla and up-regulation of Ia expression (Fig. 1 *e*), as noted previously for epidermal LC before their migration into dermis (6); this response was not observed in the renal cortex or in hearts. Although not quantified, LPS administration also resulted in loss of Ia^+ cells from liver.

Maximal loss of Ia^+ cells was induced by 50–100 μg LPS, intravenously or subcutaneously, but a reduction in numbers could be detected in kidney and heart after a single injection of 0.2 and 0.5 μg LPS, respectively (Fig. 2 *a*). Depletion was maximal 1–3 d after LPS injection, but a response was seen within 12 h (Fig. 2 *c*). The number of Ia^+ cells returned to baseline levels between 4 and 8 d after injection (Fig. 2 *c*). There were no differences in the dose response to LPS or the kinetics of Ia^+ cell depletion in C57BL/6 (Fig. 2, *a* and *c*), BALB/c (not shown), or LPS-sensitive C3H/HeN mice (Fig. 2 *b*). However, Ia^+ leukocytes were not depleted from hearts of LPS-resistant C3H/HeJ mice (Fig. 2 *b*), confirming that the response was due to LPS rather than to possible non-LPS contaminants.

Ia Loss: Modulation versus Migration. The apparent loss of Ia^+ cells from heart and kidney is unlikely to have been due to loss of Ia expression by interstitial DC (data not shown). First, Ia expression by isolated heart and kidney DC and LC was not diminished during overnight culture in medium containing LPS at up to 10 μg/ml (as shown by two-color FACS® analysis), and cell viability was not impaired. Second, there was no reduction in the number of Ia^+ presumptive interdigitating cells in cryosections of spleens or lymph nodes from treated animals, nor in the lungs or mesentery. Third, Ia was induced on renal tubular epithelium 24–48 h after LPS administration (Fig. 1 *f*), seeming to indicate that local conditions favored Ia induction rather than down-modulation. This is consistent with the known effects of inflammatory mediators, such as IFN-γ, which are induced by LPS (15–18). These observations are consistent with the hypothesis that the cytokines elicited by administration of LPS promote the migration of DC from mouse heart and kidney.

Ia Loss: Cytotoxicity versus Migration. Two lines of evidence suggest that the apparent loss of Ia^+ cells is not due to cytotoxicity. First, exposure to LPS did not result in loss of Ia^+ cells from LPS-resistant C3H/HeJ mice. This argues strongly against cytotoxicity of LPS but does not exclude

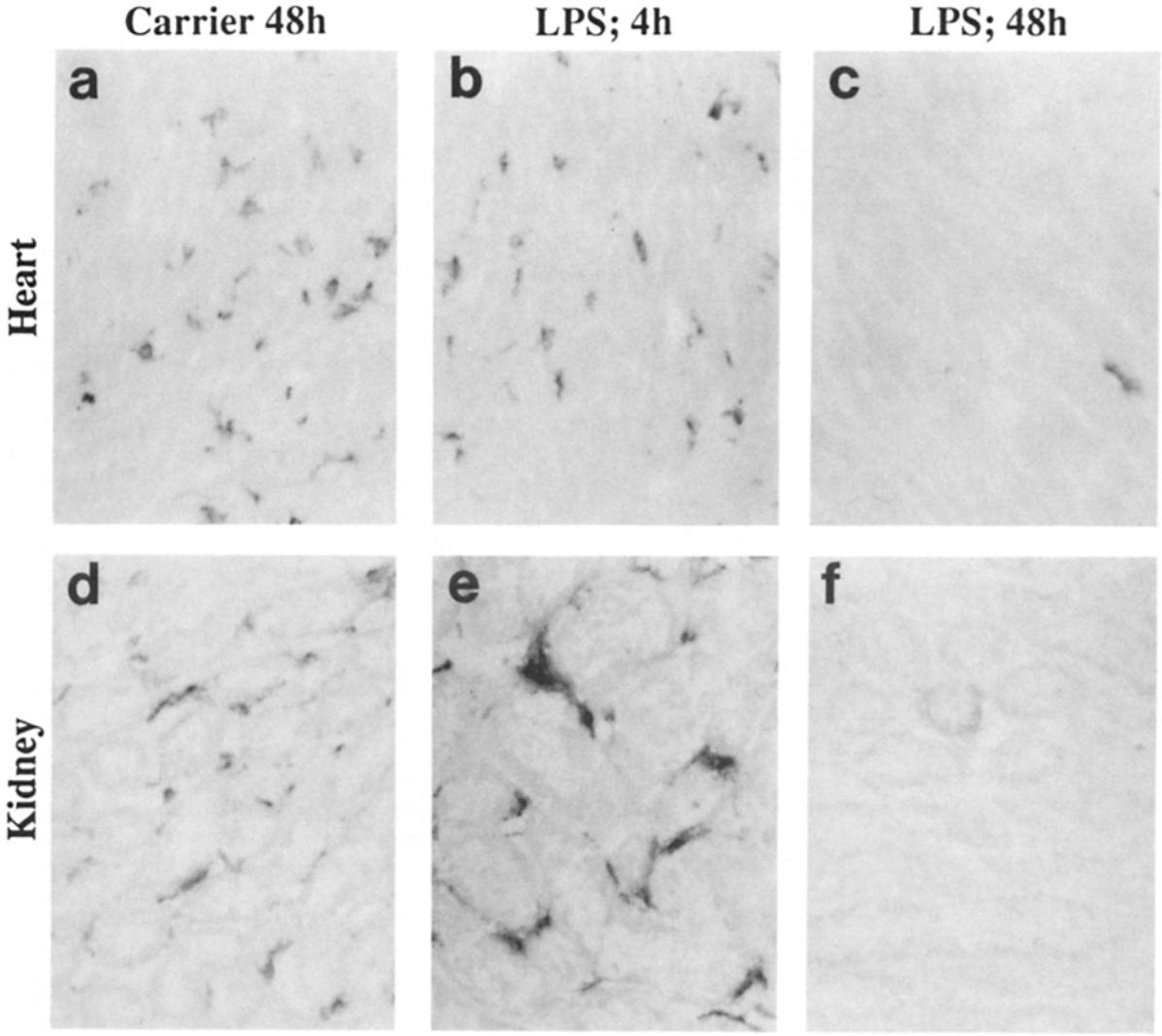

Figure 1. Depletion of Ia$^+$ cells from heart and kidney after systemic injection of LPS. Immunoperoxidase labeling of BALB/c heart (*a–c*) and kidney medulla (*d–f*) cryosections for Ia: 48 h after injection of LPS-free carrier (*a*, *d*); and 4 h (*b*, *e*), or 48 h (*c*, *f*) after a single s.c. injection of 100 μg LPS. Similar results were obtained using 50 μg LPS and in C57BL/6 mice. Note the profound depletion of Ia$^+$ cells from heart and kidney 48 h after LPS administration, which is preceded by an increase in size and up-regulation of Ia by interstitial cells in the renal medulla. The original magnification is 250.

cytotoxicity from the cytokine cascade induced by LPS in LPS-sensitive mice.

The second line of evidence is provided by transplantation experiments. It is known that Ia$^+$ donor cells can be detected in the spleen of recipients of transplanted normal hearts (4, 14) and hearts depleted of Ia$^+$ cells by LPS (14), the latter apparently due to recruitment of Ia$^-$ DC progenitors. The response to LPS was examined further in LPS-sensitive C3H/HeN recipients of allografted C57BL/6 hearts. Migratory donor Ia$^+$ cells were detected in the spleens of C3H/HeN recipients that had received 50 μg of LPS s.c. 6 h after transplantation. However, at 30 h after transplantation, the density of donor cells was not substantially increased by the administration of LPS in comparison to controls that did not receive LPS (1.57 ± 0.37, n = 3, vs 1.26 ± 0.57, n = 3, cells/mm^2; P = 0.475). This was not surprising because transplantation itself is a potent stimulus to DC migration (14), and the rate of disappearance of DC from transplanted hearts (4) is not very different than that induced by LPS. This experiment, however, provides evidence that LPS did not initiate a lethal stimulus for DC in vivo or loss of Ia expression on interstitial DC.

LPS Recruits Macrophages and Neutrophils into Mouse Heart

LPS induced a marked increase in the number of CD45$^+$ leukocytes in the heart, concomitant with the loss of Ia$^+$ cells (Fig. 2 *d*). Many of these cells expressed high levels of CD11b/CD18 type 3 complement receptors (CR3), F4/80, and CD32 Fc receptors (FcRII) (Fig. 2, *c* and *d*). After isolation as described (7), the majority of these Ia$^-$ leukocytes

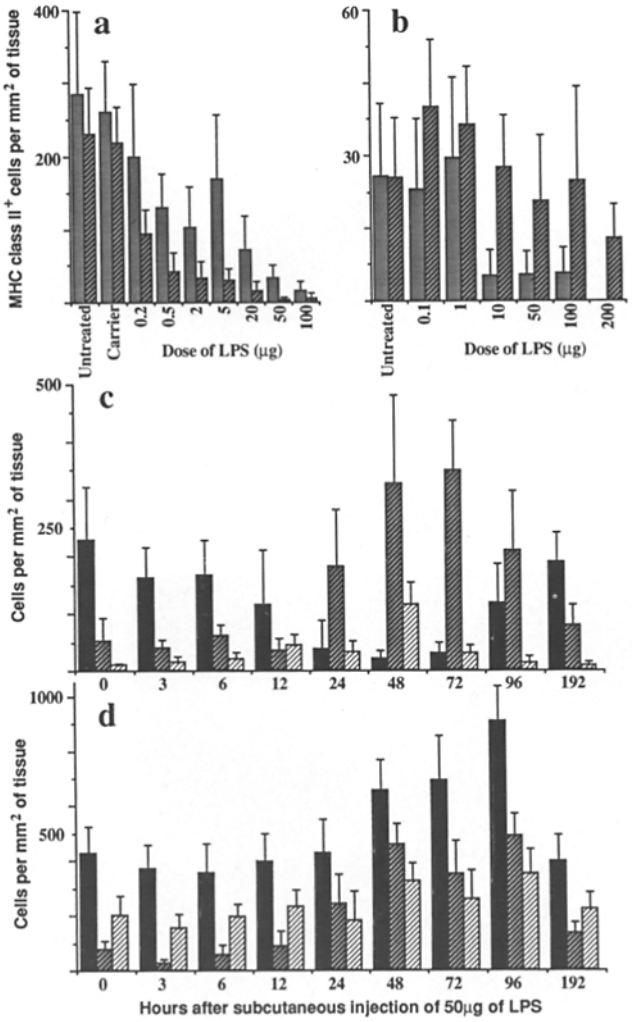

Figure 2. Dose response and kinetics of the response to LPS in heart and kidney. Data from immunoperoxidase labeling of cryosections: (*a*) heart (▩) and renal cortex (▨) of C57BL/6 mice, and (*b*) hearts of LPS-sensitive C3H/HeN (▩) versus LPS-resistant C3H/HeJ (▨) mice, labeled for Ia, 48 h after a single i.v. (*a*) or s.c. (*b*) injection of the indicated doses of LPS (similar data were obtained in *a* after s.c. injections; not shown); (*c–d*) hearts of C57BL/6 mice, labeled for (*c*) Ia (■), F4/80 (▨), and 7/4 (▨), and (*d*) CD45 (■), CD11b (CR3) (▨), and CD32 (FcR) (▨) at the indicated times after a single s.c. injection of 50 μg LPS. Each bar (*a–d*) represents the mean ± SD of counts from 5–10 high power fields from each of two animals; similar results were obtained in two experiments. Note that the content of Ia$^+$ interstital cells in C3H hearts was ~10% that of C57BL/6 or BALB/c hearts.

were cytologically identified as macrophages (data not shown). There was also a modest increase in cells expressing the 7/4 marker, which were cytologically confirmed to be neutrophils (data not shown). Similar observations were noted for kidneys of treated mice (data not shown). These findings, and the fact that DC progenitors were also recruited into these tissues (14), make it unlikely that the loss of Ia$^+$ cells was due to cytotoxicity of LPS for leukocyte stem cells.

Effects of LPS on Epidermal LC

After injection of 50–100 μg LPS, there was a reproducible decrease in the number of epidermal LC at 48 h (compare Fig. 3 *b* with *a*; see Table 1). A subset of the epidermal LC increased in size and expressed higher levels of Ia (Fig. 3 *b*). Within the dermis, "cords" of Ia$^+$ leukocytes with dendritic morphology became evident at 1–3 d, resembling in appearance and distribution those described previously (5) in skin transplants and explants (compare Fig. 3 *f* to *e*). An important difference, however, became apparent after two-color immunofluorescent labeling for Ia (or CD45; data not shown) and the DC-restricted marker NLDC145. After organ culture, dermal cords were composed exclusively of NLDC145$^+$ Ia$^+$ leukocytes of similar phenotype to the resident LC (compare Fig. 4 *a* to *d*). However, after LPS administration, NLDC145 was weak or undetectable on most Ia$^+$ leukocytes forming dermal cords (compare Fig. 4 *b* to *e*). One explanation for these findings is that systemic LPS induces migration of a subset of LC from the epidermis but recruits DC progenitors from blood to dermis or promotes their development from a resident Ia$^-$ dermal population (see also 14).

Systemic Administration of TNF-α or IL-1α, but Not IL-2, Mimics Some of the Responses to LPS

TNF-α and IL-1α are elicited after LPS injection, and both cytokines have profound effects on various DC populations in vitro (1, 2, 6). Mice were therefore treated with each of these recombinant cytokines, and cryosections were examined by immunocytochemistry 48 h later (Table 1).

TNF-α. rhTNF-α induced a marked loss of Ia$^+$ cells from heart, renal cortex, and renal medulla (Table 1). Ia$^-$ leukocytes of a similar composition (i.e., macrophages and neutrophils) to that found after LPS administration were recruited into these tissues (not shown). Epidermal LC were depleted (Table 1), and dermal cords became evident. Depletion of interstitial DC and LC was not increased after a twofold increase in dose of cytokine, indicating that a plateau was reached.

IL-1α. rhIL-1α induced a marked loss of Ia$^+$ cells from renal medulla but had no detectable effect on renal cortical or cardiac DC (Table 1). Leukocytes were recruited into these tissues, but the infiltrating cells were primarily neutrophils, with few macrophages (not shown). There was no decrease in numbers of epidermal LC (Table 1), but up to 5% of the cells increased in size and expressed higher levels of Ia, and dermal cords became evident. These effects were not increased by a twofold increase in dose of cytokine.

IL-2. None of the above effects were detectable after administration of similar microgram doses of rhIL-2 (Table 1).

We conclude that TNF-α, and to a lesser extent IL-1α, can mimic some of the responses elicited by LPS. However, these molecules function in complex cytokine cascades, and each cytokine can, in certain situations, induce production of the other.

To determine whether or not TNF-α could mediate the effects of LPS administration, studies were carried out with

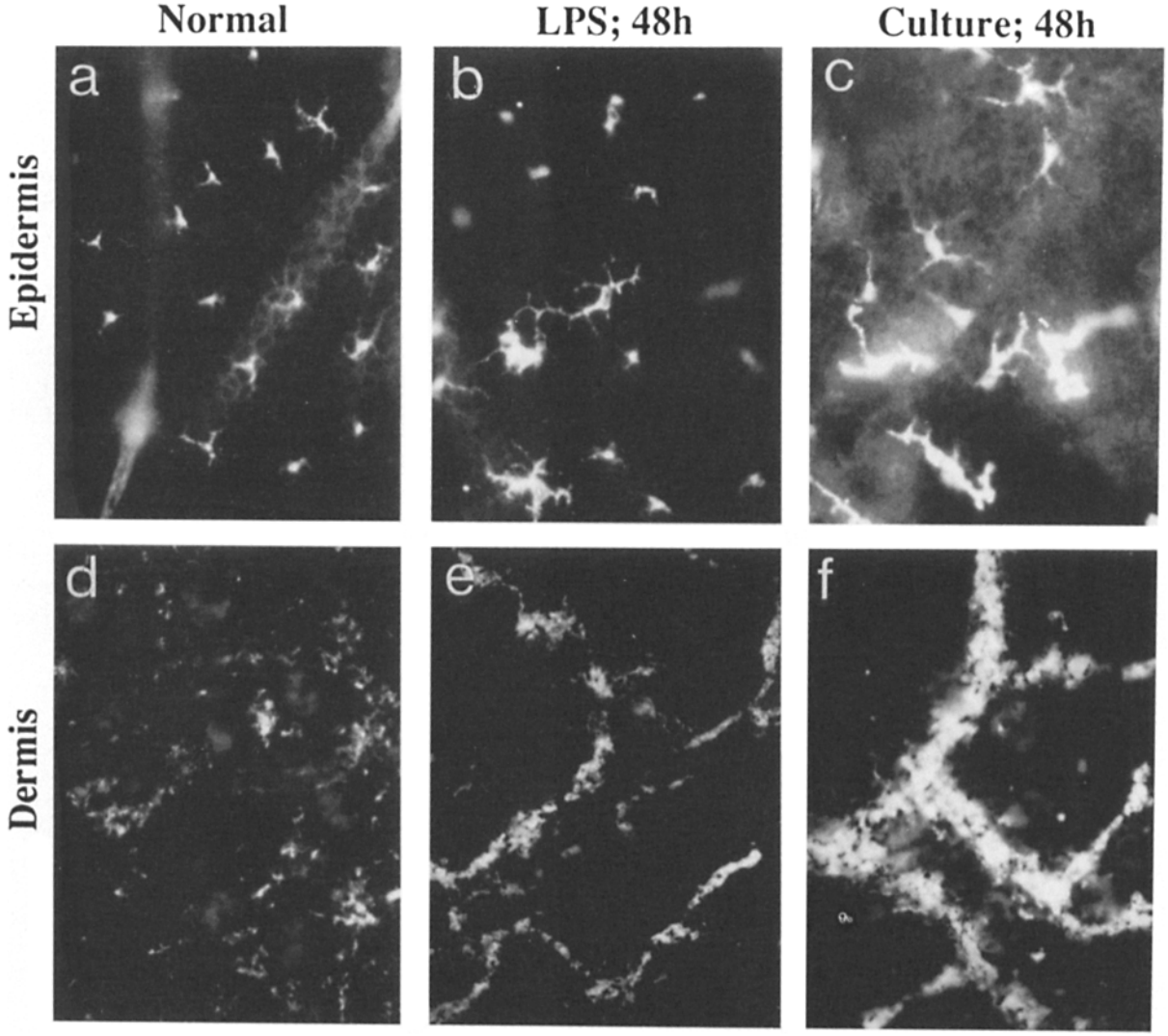

Figure 3. Ia$^+$ cells in skin after systemic injection of LPS or organ culture. Single-color immunofluorescent labeling for Ia: epidermal (*a–c*) and dermal (*d–f*) sheets prepared from normal skin (*a*, *d*) skin of mice treated 48 h previously with a single injection of 100 μg LPS s.c. (*b*, *e*), and skin explants cultured for 48 h (*c*, *f*). Original magnifications were 312 (epidermis) and 125 (dermis). The number of epidermal LC was reduced after LPS treatment (see Table 1), and a subset increased in size and expressed higher levels of Ia. Cords of Ia$^+$ cells similar to the appearance after organ culture developed in the dermis. After injection of LPS-free carrier, the appearance was similar to that of untreated normals.

neutralizing anti-TNF antibodies in vivo (Fig. 5). We first confirmed that LPS (but not saline carrier) induces a serum spike of TNF-α in C57BL/6 mice (Fig. 5 *a*) and in the C3H/HeN but not C3H/HeJ mice that were used for studies above (Fig. 5 *b*). This spike was completely neutralized by a polyclonal rabbit anti-mouse TNF antiserum, but not by control rabbit IgG, administered intravenously 1 h before LPS subcutaneously (Fig. 5 *c*); significant neutralizing activity was still present in the serum 48 h later (not shown).

Pretreatment with anti-TNF antiserum followed by LPS had no effect on loss of Ia$^+$ cardiac DC, but recruitment of 7/4$^+$ leukocytes was reduced (Fig. 5 *d*). Similar observations were made in kidneys (not shown). In skin, pretreatment with the anti-TNF antiserum prevented the reduction in numbers of epidermal LC by LPS treatment (Fig. 5 *e*), but there was no effect on dermal cord formation (not shown). The latter suggests that the cells forming these structures were either recruited to the tissue or developed from resident precursors.

We conclude that either neutralization of serum TNF-α does not reflect events at the tissue level, or mediators distinct from, or in addition to, TNF-α are involved in the response of interstitial DC to LPS.

Discussion

Our interpretation of the data is that cytokines induced by LPS in vivo are responsible for promoting migration of DC from nonlymphoid tissues. In previous studies, cells with the phenotypic and functional properties of DC resided within

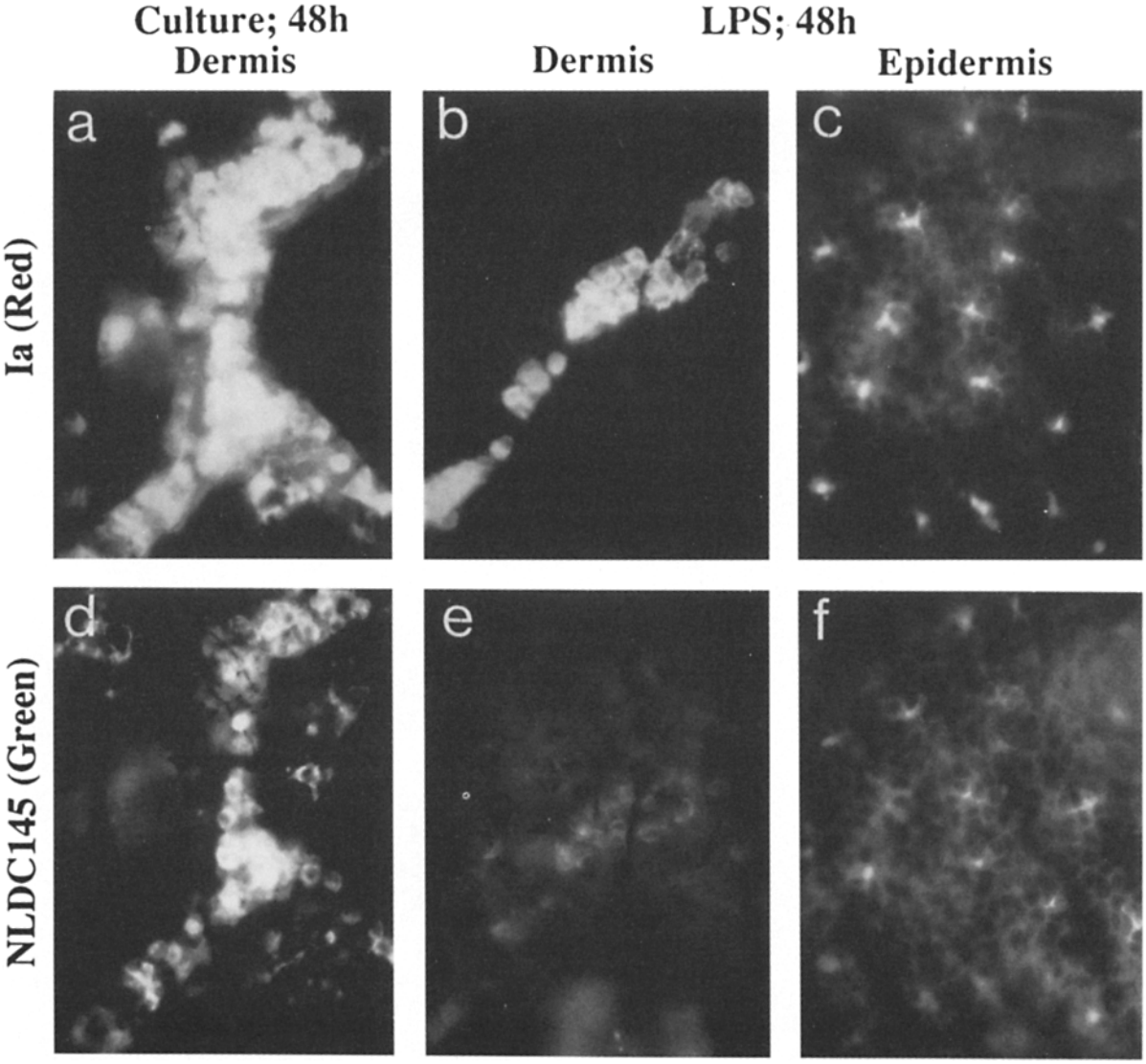

Figure 4. Ia^+ cells in skin after systemic injection of LPS or organ culture: coexpression of NLDC145. Two-color immunofluorescent labeling for Ia with Texas red (*a–c*), vs the same fields (*d–f*) labeled for NLDC145 with FITC: dermal (*a*, *b*, *d*, and *e*), and epidermal (*c*, *f*) sheets were prepared from skin explants cultured for 48 h (*a*, *d*), and skin of mice treated 48 h previously with a single injection of 100 μg LPS s.c. (*b*, *c*, *e*, and *f*). The original magnifications were 400 (dermis) and 500 (epidermis). Dermal cords induced by LPS treatment were composed of Ia^+ but $NLDC145^-$, or weakly positive, leukocytes (CD45 labeling not shown), whereas these cells expressed both markers in skin explants. In the epidermis after injection of LPS, and in normal skin and skin explants (not shown), all Ia^+ cells expressed NLDC145, suggesting that LPS had not simply modulated expression of NLDC145 by the Ia^+ cells in the dermis.

the Ia^+ subset of leukocytes isolated from mouse heart and kidney (7). It is difficult to determine whether or not macrophages also expressed Ia, but at least those recruited to the tissues by systemic LPS were Ia^- cells (Fig. 2 *c*). It therefore seems reasonable to assume that the profound reduction in numbers of Ia^+ cells induced by LPS relates at least in part to interstitial DC, although this is less clear for responses to systemic cytokines.

There are three possible explanations for the response to LPS: cell migration, loss of Ia expression, or cytotoxicity. It is unlikely that the loss of Ia^+ cells was due to loss of Ia or a cytotoxic effect for the following reasons. First, when DC were isolated and cultured in the presence or absence of LPS, there were no differences in Ia expression or cell viability. Second, presumptive interdigitating cells were readily detectable in secondary lymphoid tissues of LPS-treated mice, and Ia^+ presumptive DC were present in the lungs. It is notable that administration of LPS has in fact been suggested to increase the numbers of DC in these tissues (19, 20). Third, LPS did not induce loss of Ia^+ cells in LPS-resistant mice or loss of donor Ia^+ cells in the spleen of LPS-sensitive recipients of heart transplants. Fourth, LPS recruited DC progenitors to heart and kidney (14), and perhaps also to the dermis of skin. Despite this, it is difficult to demonstrate migra-

Table 1. *Depletion of Ia⁺ Interstitial Cells from Heart and Kidney and of LC from Epidermis after Systemic Administration of LPS, Recombinant TNF-α, IL-1α, or IL-2 to C57BL/6 Mice*

			Control			Test			Control vs test	
	Analysis	Agent	Number*	Median	Range	Number	Median	Range	Percent change°	*P*
Heart										
	Cell count	LPS‡	4	199	173–240	10	4.8	0.0–7.6	98 ↓	<0.001
	Cell count	TNF-α§	6	185	178–371	5	102	92–130	45 ↓	0.012
	Cell count	IL-1α‖	6	220	168–328	6	212	105–284	↔	0.487
	Cell count	IL-2¶	2	202	184–220	2	191	188–194	↔	
Kidney										
Renal cortex	Cell count	LPS	6	230	184–248	6	5.3	2.1–32.0	98 ↓	<0.001
	Cell count	TNF-α	6	228	192–285	5	126	112–210	45 ↓	0.005
	Morphometry	LPS	2	25.3	25–25.5	8	1.3	0–5	95 ↓	0.001
	Morphometry	TNF-α	2	17.3	13–21.5	4	8	6–9	54 ↓	0.029
	Morphometry	IL-1α	4	9	7–11	6	8	2.5–12	↔	0.567
	Morphometry	IL-2	2	15.8	14–17.5	2	16	16–16	↔	
Renal medulla	Morphometry	LPS	2	46.8	46.5–47	7	1	0–9.5	98 ↓	<0.001
	Morphometry	TNF-α	2	27	22.5–31.5	4	4.3	3–8.5	84 ↓	0.003
	Morphometry**	IL-1α	4	17	15.5–17	6	6.8	1.5–11	60 ↓	0.001
	Morphometry	IL-2	2	41	43–39	2	32.3	32–32.5		
Skin										
Epidermis	Cell count	LPS	4	523	448–568	4	403	383–419	23 ↓	0.006
	Cell count	TNF-α	4	473	509–458	5	406	436–370	14 ↓	0.002
	Cell count	IL-1α	6	483	450–548	6	501	397–535	↔	0.977
	Cell count	IL-2	2	488	486–489	2	502	539–463	↔	

Cryosections of heart and kidney, and epidermal sheets, were prepared from C57BL/6 mice 48 h after a single injection of LPS, cytokine (*Test*), or the appropriate carrier solution (*Control*), and were labeled for expression of Ia. The tissues were then analyzed by direct counting of positively labeled cells or by morphometry. Cell count values represent the mean number of cells per square millimeter of tissue. Morphometry, used for the kidney analysis, produced quite large variations between experiments, because of variations in the intensity of labeling and variability between batches of mice, but it reliably detected differences between treatment and control groups within individual experiments (compare renal cortex cell counts and morphometry after administration of LPS or TNF-α). Treatment with carrier solutions produced no significant differences from untreated animals.

* Number of animals.

° Arrows indicate a decrease (↓), increase (↑), or no change (↔).

‡ The minimum LPS dosage selected was that required to produce maximum effect based upon dose–response studies up to 100 μg (i.e., ≥50 μg for heart; ≥25 μg for kidney; 100 μg for epidermis). Similar results were obtained in more than five experiments.

§ TNF-α produced no significant effect at doses ≤2 × 10^5 U (8 μg). Combined data presented from two independent experiments after administration of 8 × 10^5 or 1.6 × 10^6 U.

‖ Dose–response studies (0.1–50 μg) indicated that the observed effects of IL-1α plateaued at ≤10 μg. Heart and skin data presented followed administration of 10 μg IL-1α. Kidney data followed administration of 10–50 μg IL-1α. Similar results were obtained in three independent experiments.

¶ The IL-2 data are from a single experiment included as a negative control for the administration of human cytokine. 10 μg of IL-2 was administered i.p. The sample size precludes meaningful statistical analysis.

** Data from both untreated and carrier-treated controls are included to facilitate the statistical analysis using the Student's *t* test.

tion of DC directly in this model. However, in a rat model, LPS administration resulted in an increased flux of veiled cells in pseudoafferent lymph that apparently derived from the gut wall (21).

We assume that LPS does not act directly on DC, but that, as in other experimental models, its effects are mediated via cytokines (15–18). In these studies, rhTNF-α and rhIL-1α were used to determine whether these cytokines can promote DC migration. Although many cytokines, including IL-1α and IL-2, appear to be fully cross-reactive between mouse and human, and mouse TNF appears to be fully active on human cells (22), human TNF is active only in some murine bioassays (23–25) and is less toxic than rmTNF-α to mice in vivo (23). Two distinct TNF receptors have been identified in the mouse: TNF-R2, which may be species specific, and TNF-R1, which is not (26). Since TNF-R1 appears to be

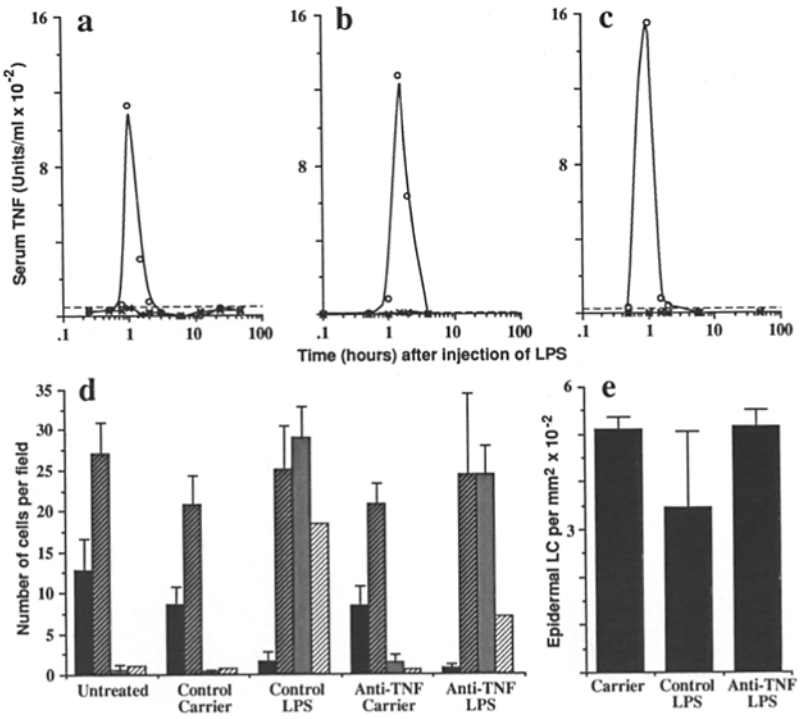

Figure 5. The effects of a neutralizing anti-TNF antiserum before LPS treatment. (*a–c*) Serum TNF levels were measured at the indicated times after injection of: (*a*) 25 μg LPS s.c. (*open circles*) or saline carrier s.c. (*crosses*) to C57BL/6 mice; (*b*) 50 μg LPS s.c. to C3H/HeN (*open circles*) or C3H/HeJ (*crosses*) mice; and (*c*) 2 × 10^4 neutralizing units of anti-TNF (*crosses*) or an equivalent microgram dose of control rabbit IgG (*open circles*) i.v., 1 h before a challenge with 25 μg LPS s.c. Each point represents the mean of duplicate assays on serum from each of two animals; the limit of detection in each L929 assay is indicated by the dashed horizontal line. LPS induced a serum TNF spike in C57BL/6 (*a*) and C3H/HeN (*b*) mice, but not C3H/HeJ mice, that was completely neutralized by prior administration of an anti-TNF antiserum. The TNF spike induced by 100 μg LPS s.c. was also completely neutralized by the same dose of antiserum, and significant neutralizing activity was still present in serum 48 h later (data not shown). (*d*, *e*) C57BL/6 mice were treated as indicated: anti-TNF or control IgG was administered (as in *a–c*) before a single s.c. injection of 25 μg (*d*) or 100 μg (*e*) LPS or saline carrier. After 48 h, heart cryosections (*d*) and epidermal sheets (*e*) were labeled for Ia (■), CD45 (■), F4/80 (■), or 7/4 (▨). Each bar represents the mean ± SD of cell counts from a minimum of five high power fields (Ia: *d*, ×500, *e*, ×625; CD45: ×500; F4/80: ×312; 7/4: ×312) of tissues from each of four animals (the data for 7/4 was not normally distributed so error bars are not shown). Similar results were obtained in two experiments. In separate experiments, administration of polyclonal anti-TNF or control IgG alone had no effect on cells in skin epidermis or dermis (data not shown). Neutralization of the serum TNF spike (see *a–c*) induced by LPS had no effect on depletion of Ia^+ interstitial cells from heart (*d*), but did prevent the reduction in numbers of epidermal LC (*e*; $p = 0.05$, Mann-Whitney test, pooled data of two experiments, $n = 8$); had no effect on recruitment of the macrophages but did reduce neutrophil recruitment to heart (*d*; $p = 0.036$, Mann-Whitney test, pooled data of two experiments, $n = 8$); and had no effect on the induction of dermal cords (semiquantitative data not shown; $P = 0.4$, Mann-Whitney test, pooled data of two experiments, $n = 8$).

the major signal transduction component for TNF responses (27), it seems likely that the responses to human and mouse TNF-α would be qualitatively (if not quantitatively) similar.

Administration of rhTNF-α led to a reduction in the numbers of cardiac and renal Ia^+ cells and of epidermal LC. In other studies, intradermal injection of rmTNF-α into ear skin of mice resulted in increased numbers of DC in draining lymph nodes (28) and depletion of epidermal LC by up to 25% 30 min after injection (29), providing evidence that TNF can promote LC migration from the skin. TNF has also been implicated in the migration of LC to lymph nodes in response to UV-B irradiation (30), although it has also been reported that UV-B irradiation or intradermal TNF may transiently immobilize LC within the epidermis (31). Treatment of mice with anti-TNF antiserum before LPS administration was sufficient to prevent the depletion of LC from the epidermis but not of cardiac or renal DC. Interpretation of these observations is difficult because, even though TNF was neutralized within the serum, this might not have been the case within the interstitial spaces of heart and kidney. In terms of other cytokines, it has been reported that intraperitoneal injection of IFN-α or IFN-β leads to loss of Ia^+ cells from mouse kidney and heart (32).

Administration of rhIL-1α led to a decrease in the number of Ia^+ cells in the renal medulla, but, in contrast to TNF, it did not decrease the number of Ia^+ cells in the renal cortex or heart, nor of epidermal LC (Table 1). The latter is consistent with the finding of Enk et al. (33) that IL-1β, but not IL-1α, can induce migration of epidermal LC. In other experiments, systemic or intracutaneous injection of IL-1β was reported to decrease the density of epidermal LC by up to 50% between 2 and 7 d after injection (34), but injection of IL-1 into the central cornea induced centripetal migration of peripheral corneal LC to the site of injection (35).

In summary, the data in the present report indicate that systemic LPS, TNF-α, and IL-1α can promote the migration of different populations of DC, either directly or indirectly (e.g., by inducing other cytokines). However, given the pleiotropic and apparently degenerate nature of cytokine activity in general, it may prove very difficult to define individual cytokines that mediate DC migration or recruitment directly. A role for inflammatory mediators in promotion of nonlymphoid DC migration may be physiologically and therapeutically relevant, but it is not clear if local exposure to physiologically relevant concentrations of LPS during bacterial infection would induce local DC migration and leukocyte recruitment. It is tempting, however, to suggest that this may be a component of the early host response to bacterial invasion.

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