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*J Clin Invest.* 2006;116(12):3160-3170. [https://doi.org/10.1172/JCI28996](https://doi.org/10.1172/JCI28996).

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Indoleamine 2,3-dioxygenase–expressing dendritic cells form suppurative granulomas following *Listeria monocytogenes* infection

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Control of pathogens by formation of abscesses and granulomas is a major strategy of the innate immune system, especially when effector mechanisms of adaptive immunity are insufficient. We show in human listeriosis that DCs expressing indoleamine 2,3-dioxygenase (IDO), together with macrophages, are major cellular components of suppurative granulomas in vivo. Induction of IDO by DCs is a cell-autonomous response to *Listeria monocytogenes* infection and was also observed in other granulomatous infections with intracellular bacteria, such as *Bartonella henselae*. Reporting on our use of the clinically applied anti–TNF-α antibody infliximab, we further demonstrate in vitro that IDO induction is TNF-α dependent. Repression of IDO therefore might result in exacerbation of granulomatous diseases observed during anti–TNF-α therapy. These findings place IDO+ DCs not only at the intersection of innate and adaptive immunity but also at the forefront of bacterial containment in granulomatous infections.

Introduction

Restriction of parenchymal and systemic spreading of intracellular bacteria, such as *Mycobacterium tuberculosis* or *Listeria monocytogenes*, is achieved by granulomatus structures or abscesses containing cellular and acellular components. The major role in this process has been attributed to an intricate cooperation among macrophages, neutrophils, and T cells (1–3). Listeria infection in mice has been exploited extensively as a model to study molecular mechanisms of early innate as well as adaptive immunity (4, 5). During systemic *L. monocytogenes* infection in mice, macrophages are central to the early innate immune response, acting as both hosts for and major defenses against this pathogen (6, 7). Macrophages have also been the major cell type used in the analysis of cell-autonomous defense mechanisms that these cells had to evolve to counterbalance microbial attack (2, 8, 9).

For entry into host epithelial cells, *L. monocytogenes* requires interaction of the bacterial surface protein internalin A with E-cadherin while internalin B binds to the hepatocyte growth factor receptor (Mer), the receptor for the globular head of the complement factor C1q (gC1qR), and glycosaminoglycans (10). Humans are mainly infected with *L. monocytogenes* via the gastrointestinal tract (foodborne disease) while in mice, enteral infection is not efficient due to a single amino acid substitution in murine E-cadherin (11, 12). Healthy humans usually clear *L. monocytogenes* infection with little or no clinical symptoms. However, in patients with predisposing conditions, such as diabetes mellitus, liver failure, HIV infection, immune suppression, splenectomy, older age (> 75 years), and pregnancy, or undergoing anti–TNF-α therapy, listeria can cause a potentially life-threatening disease with clinical symptoms ranging from local inflammatory responses to meningoencephalitis, sepsis with suppurative granulomas in multiple organs, or devastating maternal/fetal infection in pregnant women (13, 14). In these patient populations, the incidence is as high as 210 cases per 100,000 (as compared with 0.7 per 100,000 cases in healthy individuals), and mortality can reach 30% (13).

A major role of DCs during early enteral *L. monocytogenes* infection has been recently suggested by immunohistochemical studies in rats (15). DCs phagocytose *L. monocytogenes* in the small intestine after the bacteria cross the intestinal barrier and are the major cells transporting *L. monocytogenes* to the draining mesenteric lymph nodes. DCs have to manage a delicate balance. On one hand, they have to allow the escape of *L. monocytogenes* from phagosomes and enable subsequent replication in the cytosol in order to promote delivery of *L. monocytogenes* antigens into the MHC class I–restricted antigen processing and presentation pathways. This is essential for induction of CD8+ CTL, a prerequisite for sterile elimination of *L. monocytogenes* (3, 16–18). On the other hand, intracellular replication must be tightly controlled in order to prevent destruction of DCs, which would result in both lack of CTL induction and systemic spreading of bacteria. Thus, DCs had to evolve mechanisms controlling the number of viable intracellular *L. monocytogenes* (15, 19, 20). For at least one subtype of murine DCs, production of TNF-α and iNOS has been implicated in innate immune functions of DCs (21). Human and murine immature DCs (immDCs) efficiently internalize *L. monocytogenes* in vitro, which is followed by rapid DC maturation accompanied by secretion of cytokines,

Nonstandard abbreviations used: CSD, cat-scratch disease; EIA, enzyme-linked immunosassay; hk, heat killed; IDO, indoleamine 2,3-dioxygenase; immDC, immature DC; LTA, lipoteichoic acid; PGEl, prostaglandin E1; rhIDO, recombinant human IDO.

Conflict of interest: The authors have declared that no conflict of interest exists.

including IL-12 or IL-18, the latter inducing neutrophil activation and migration. In vitro, these DCs can induce significant T cell proliferation (17, 22, 23).

These findings suggest that DCs, in addition to macrophages and neutrophils, play a role during early steps of innate immunity to enteral L. monocytogenes infection. While many aspects of early host-pathogen interactions have been described, surprisingly little is known about the cellular components and mechanisms responsible for the formation of granulomas during advanced-stage listeriosis in patients with impaired adaptive immunity.

Here we show for what we believe is the first time that DCs, along with macrophages, are a major cellular component of the outer ring wall of suppurative granulomas in human listeriosis. Moreover, DCs and macrophages within the ring wall strongly express the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO). With in vitro studies of human DCs, we show that IDO induction is TNF-α and IFN-γ dependent following infection of DCs with L. monocytogenes.

**Results**

IDO+ DCs are a major component of granulomas in listeriosis. Lymphadenopathy is a hallmark of advanced listeriosis and is characterized by specialized suppurative granulomas with typical ring-wall formation (24). To better understand the cellular reactions involved in this process, we studied the components of the abscesses in lymph node specimens of 3 patients with serologically confirmed cervico-glandular–type infection with L. monocytogenes. Bacterial genes were verified in the diseased lymph nodes by PCR (Supplemental Table 1 and Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI28996DS1). H&E staining revealed large numbers of the typical granulomas with ring-wall formation accompanied by follicular and interfollicular hyperplasia (Figure 1A). Using markers specific for B cells (CD20) and T cells (CD3), we demonstrated that these specialized cells of the adaptive immune response are not part of granulomas in listeriosis but are almost exclusively located in between granulomas. Since macrophages and neutrophils are known to play major roles in systemic listeriosis in murine models (8, 25, 26), we next assessed expression of CD15 (granulocyte marker) (27) and CD68 (macrophage marker) (28) in infected tissues (Figure 1A). Within the center of the granulomas, basically all cells were CD15+ granulocytes, with only a few CD68+ macrophages. The number of CD68+ cells within the ring wall was variable among different granulomas in the same lymph node specimen, ranging from 30% CD68+ cells up to 70% of all cells forming the ring wall. Unexpectedly, there was a high number of S100+ DCs (29) within this area while only a few DCs were found within the center of the granulomas or outside the ring-wall structure (Figure 1A). The clear separation of T and B cells in the
genes are clearly distinguish between DCs and macrophages. First, double-staining for S100 and CD68 confirmed that these tumors, 2-color immunofluorescence was performed (Figure 1B).

Figure 1A, the great majority of cells within the cellular ring wall were strongly positive for IDO. We therefore assessed IDO expression in listeriosis. As shown in Figure 1B, basically all S100+ cells expressed significant levels of IDO whereas some smaller cells in between were IDO–S100+. Double staining using IDO and CD11c revealed that all IDO+ cells were CD11c+, suggesting that the small S100–IDO+ cells could be macrophages. Indeed, double staining of IDO and CD68 confirmed that macrophages also expressed IDO, although to a lesser extent than DCs. Moreover, some CD68+ macrophages did not express IDO. Colocalization of IDO with S100 or CD68 was clearly confirmed by confocal microscopy (Figure 1C). In summary, in addition to CD68+ macrophages, large myeloid CD11c+IDO–S100+CD68–DCs are a major cell population contributing to the ring-wall structure of granulomas in human listeriosis.

Staining with a specific antibody to the myeloid marker CD11c revealed that virtually all S100+ DCs were of myeloid origin (31). As expected, CD68+ macrophages were also CD11c+ positive and were clearly smaller than S100+ DCs. Next we allocated IDO expression to both cell populations. As can be seen in Figure 1B, basically all S100+ cells expressed significant levels of IDO whereas some smaller cells in between were IDO–S100+. Double staining using IDO and CD11c revealed that all IDO+ cells were CD11c+, suggesting that the small S100–IDO+ cells could be macrophages. Indeed, double staining of IDO and CD68 confirmed that macrophages also expressed IDO, although to a lesser extent than DCs. Moreover, some CD68+ macrophages did not express IDO. Colocalization of IDO with S100 or CD68 was clearly confirmed by confocal microscopy (Figure 1C). In summary, in addition to CD68+ macrophages, large myeloid CD11c+IDO–S100+CD68–DCs are a major cell population contributing to the ring-wall structure of granulomas in human listeriosis.

Upregulation of IDO by immature DCs is an early but not immediate event. To examine the significance of in vivo IDO expression in DCs, we used the well-established in vitro system of monocytederived myeloid immdCs (23, 32, 33) and assessed genome-wide transcriptional changes in immdCs 2 hours and 6 hours after infection with L. monocytogenes (for further details, see Supplemental Data and Supplemental Figure 2), using Affymetrix HG-U133A oligonucleotide microarrays. Unsupervised hierarchical clustering of samples based on the 1189 most variable transcripts (Supplemental Data) clearly distinguished infected immdCs from controls (Figure 2A). Within the group of infected immdC samples, the 2 time points were segregated, suggesting differential kinetics of transcriptional changes after infection with L. monocytogenes. We observed 3 patterns of transcriptional changes after infection similar to those found in murine bone-marrow–derived macrophages (34), which we termed immediate (changes only at 2 hours), immediate-early (changes at 2 and 6 hours), and early responses (changes at 6 hours) (Figure 2B, Supplemental Table 2, and Supplemental Data). IDO mRNA was undetectable in all control samples and after 2 hours of infection but significantly upregulated (37-fold,
IDO expression was assessed by quantitative real-time PCR. Expression of β2 microglobulin (B2M) was used as a housekeeping gene control. Shown here are IDO expression profiles (normalized to B2M expression) in DC cultures derived from 3 different donors. Samples after L. monocytogenes infection are represented by filled symbols, the corresponding control samples by open symbols. (B) Protein expression of IDO and β-actin was assessed by immunoblotting after L. monocytogenes infection. Results of 1 representative experiment out of 6 are shown. rhIDO was used as a positive control. (C) Tryptophan depletion by enzymatically active IDO in cell supernatants was assessed by reverse-phase HPLC. Shown here is the reduction of tryptophan after 6, 12, or 24 hours in DC culture supernatants relative to tryptophan concentrations measured in DC medium alone. Mean ± SD of 3 independent experiments is shown. Asterisks highlight statistically significant comparisons (*** P < 0.001, ** P < 0.01). (D) Kynurenine accumulation at the same time points was assessed using a photometric assay. Shown here are mean ± SD of 3 independent experiments. Asterisks highlight statistically significant comparison (** P < 0.01). (E) Macrophages (Mφ) and DCs were differentiated from monocytes and infected with wild-type L. monocytogenes. After 24 hours, IDO protein expression was assessed by immunoblotting and analyzed quantitatively respective to β-actin expression. Representative Western blot and mean ± SD of 3 independent experiments are shown. Asterisks highlight statistically significant comparison (** P < 0.01).

Figure 3

IDO is expressed on transcriptional and functional levels in human DCs infected with L. monocytogenes. IDO is expressed on transcriptional and functional levels in human DCs infected with L. monocytogenes. To assess whether differential expression of IDO between CD11c+CD68+ macrophages and S100+CD11c+ DCs in vivo is also reflected by different levels of IDO in vitro, we generated macrophages and DCs from monocytes derived from 3 healthy individuals and infected these cells with L. monocytogenes. As shown in Figure 3E, IDO expression in the in vitro–generated DCs was also higher than in the in vitro–generated macrophages. This was also reflected by lower levels of IDO mRNA in macrophages (data not shown). Even with prolonged culturing (up to 48 hours), infected macrophages did not show enhanced IDO protein expression (data not shown). Regulation of TNF-α and COX-2 precedes IDO expression after infection. Type I and II IFNs were previously described as potent inducers of IDO in macrophages (37), HeLa cells (38), and placentae (39). More recently, it was demonstrated that TNF-α, together with prostaglandin E2 (PGE2), can also induce IDO in DCs (33, 40). We therefore evaluated transcriptional changes for IFNs, TNF-α, COX-2, and one of the key enzymes of PGE2 synthesis (PGE2 synthase; ref. 41), and their receptors (Figure 4A and Supplemental Table 3). Transcripts for TNF-α (TNF) and COX-2 (PTGS2) were already significantly upregulated at 2 hours after infection and further increased by 6 hours after infection. In contrast, transcripts coding for IFNs (IFNG, IFNBI) were only upregulated 6 hours after infection. Interestingly, among the respective receptors, only the PGE2 (EP4 or PTGER4), known to bind PGE2 on DCs (42), and TNFRSF1B (TNF-R1I), p75 TNF receptor, which predominantly
binds the transmembrane form of TNF (43), were significantly upregulated (Figure 4A and Supplemental Table 3).

Next we assessed protein expression of TNF-α, IFNs, and COX-2 as well as PGE2 synthesis. While none of the molecules were expressed in control cultures, TNF-α was detected in substantial amounts in supernatants from infected immDCs as early as 2 hours after infection and further increased at later time points (up to 60 ng/ml; Figure 4B). Similarly, COX-2 was already expressed at low levels 2 hours after infection (Figure 4C) and increased significantly by 6 hours after infection and by more than 10-fold up to 24 hours after infection, as determined by quantitative analysis of immunoblots. Moreover, activation of COX-2 immediately after infection resulted in the early onset of PGE2 production, which was revealed by measuring PGE metabolites by enzyme-linked immunoassay (EIA) in culture supernatants (Figure 4D). In contrast, IFN-γ was measurable just above background levels at 6 hours after infection and remained undetectable by ELISA in the supernatants throughout the culture period (data not shown). Interestingly, the in vitro-infected human macrophages secreted similar levels of TNF-α and IFN-γ when compared to DCs (data not shown). These data clearly demonstrate that TNF-α and COX-2 precede the induction of IDO while a low level of IFN-γ is detectable concomitantly with IDO at 6 hours with moderate upregulation at 24 hours after infection.

Neutralization of TNF-α or IFN-γ reduces expression and activity of IDO. The kinetics of TNF-α and PGE2 induction during L. monocytogenes infection suggested that they, along with IFN-γ, might be mediating the upregulation of IDO (33, 40). To address this issue, we performed blockage experiments using a concentration range of the clinically used TNF-α-neutralizing antibody infliximab (Remicade, 0.1–10 μg/ml), the COX-2 inhibitor rofecoxib (Vioxx), and research-grade blocking antibodies for IFN-γ and IFN-β at neutralizing concentrations. Inflimximab significantly impaired upregulation of IDO mRNA in a concentration-dependent manner after L. monocytogenes infection of immDCs (Figure 5A). Similarly, anti–IFN-γ also inhibited upregulation of IDO mRNA while both rofecoxib and anti–IFN-β had no effect. Interestingly, the combination of infliximab and anti–IFN-γ antibodies did not further suppress IDO upregulation (data not shown), suggesting that both factors might be involved in the same pathway. Similarly to transcriptional regulation, neutralization of TNF-α and IFN-γ, but not COX-2 or IFN-β, significantly inhibited the expression of IDO protein (Figure 5B). Furthermore, IDO enzymatic activity was also significantly decreased by TNF-α or IFN-γ blockade, as was reflect- ed by significantly reduced tryptophan depletion (data not shown) and kynurenic acid accumulation (Figure 5C) in cell culture supernatants. Analysis of TNF-α and IFN-γ in culture supernatants revealed that TNF-α blockade also inhibited IFN-γ upregulation in a dose-dependent manner (Figure 5D) while IFN-γ blockade did...
not impair TNF-α induction (Figure SE), placing IFN-γ induction downstream of TNF-α in human DCs after L. monocytogenes infection. Combining TNF-α blockade with COX-2 inhibition did not further reduce IDO function, suggesting that PGE₂ is not required for induction of IDO activity after L. monocytogenes infection (data not shown). Neutralization of IFN-β had no influence on either IDO expression or on its enzymatic activity (Figure 5).

**Major virulence factors of L. monocytogenes are not required for induction of IDO.** To determine which host-pathogen interactions might be critical for the induction of IDO in immDCs infected with listeria, we used 2 well-characterized avirulent L. monocytogenes mutants (Δhly and prfA) as well as heat-killed (hk) bacteria. The Δhly mutant does not allow escape of L. monocytogenes from phagosomes into the cytosol due to the defect of listeriolysin synthesis (44) while the prfA mutant lacks most virulence factors due to the impairment of the major listerial virulence regulator PrfA (45). Immunofluorescence microscopy of DCs infected with FITC-labeled L. monocytogenes mutants and stained for F-actin revealed no typical actin condensation (up to 2 hours after infection), indicating that avirulent L. monocytogenes did not escape from the phagosome (data not shown). As an additional control, hk L. monocytogenes, which are phagocytosed by DCs but are quickly destroyed within phagosomes were used. As shown in Figure 6A, the 2 mutant listeria strains induced functionally active IDO, similar to the induction in wild-type L. monocytogenes. However, hk listeria did not induce detectable levels of IDO protein or its enzymatic activity. Likewise, COX-2 expression and PGE₂ synthesis were strongly induced by the 2 mutants but not by phagocytosed hk L. monocytogenes (Figure 6B). The lack of induction of IDO and COX-2 by hk bacteria was accompanied by only low-level secretion of TNF-α at all time points measured (Figure 6C). Analysis of genome-wide transcriptional changes over time further demonstrated that hk listeria did not lead to a sustained response of immDCs (Figure 6D). In summary, while IDO is induced by viable L. monocytogenes mutants lacking well-described virulence factors, challenge of DCs with hk listeria does not upregulate TNF-α, COX-2, and IDO and is accompanied by an overall limited transcriptional response.

To elucidate potential factors expressed by viable listeria responsible for the induction of IDO, we postulated such a factor to be involved in early host-pathogen interactions between bacterial surface proteins and the pattern recognition receptors of DCs, such as TLR. LPS, a gram-negative bacteria-derived TLR ligand, has recently been shown to be involved in upregulation of IDO in myeloid DCs (40); however, gram-positive listeria do not express LPS. The cell wall of listeria consists mostly of lipoteichoic acid (LTA) (46, 47), which utilizes TLR2 as a signaling receptor (48). Interestingly, expression of TLR2 on DCs was significantly upregulated during listeria infection (Supplemental Table 2). We therefore hypothesized that LTA might be involved in the induction of IDO. Assessment of IDO expression in DCs stimulated with highly purified LTA derived from L. monocytogenes revealed that LTA can indeed induce IDO expression in DCs (Figure 6E).
infection since no other exogenous. Transcrip

Figure 6
Influence of L. monocytogenes virulence factors on IDO induction. immDCs were either infected with the listeria mutants Δhly or prfA or incubated with hk L. monocytogenes for 30 minutes, washed, and subsequently cultured for 2, 6, or 24 hours Alternatively, DCs were treated with purified LTA derived from L. monocytogenes and cultured for 72 hours. Cells and supernatants were then harvested to assess (A) IDO protein expression and tryptophan levels, (B) COX-2 protein expression and PGE metabolite levels, and (C) TNF-α concentration. Mean ± SD from 2 experiments are shown. Asterisk highlights statistically significant comparison (*P < 0.05). Immunobots are representative of 2 independent experiments. (D) A heat map illustrating the kinetics of gene expression in immDCs treated with hk L. monocytogenes or infected with virulent L. monocytogenes (WT) and corresponding mock-infected controls on a Sentrix Human-6 Expression BeadChip array. Examination of genes showing significant differences in expression levels between control and listeria-infected DCs at 1 of the 3 time points (fold change > 2; absolute difference in signal intensity > 100) yielded 1,444 candidate genes. Absolute expression values of these gene transcripts in all 3 cell subsets were color coded (white, low expression; dark red; high expression); scale of expression values ranged from 0 to 50,000. (E) IDO protein expression in human DCs was assessed by immunoblotting after 72 hours of LTA treatment at indicated concentrations; β-actin was used as loading control and nlIDO as a positive control. Results of 2 representative experiments are shown.

Discussion
Formation of abscesses or granulomas is an important host reaction to control pathogens, especially when the latter cannot be eliminated by an effective adaptive immune response. The cellular components and molecular mechanisms involved are strongly dependent on the particular pathogen and the host responsiveness. Most often, macrophages and T cells have been associated with ring-wall formation surrounding such containment structures (1–3). Here we demonstrate that DCs, along with macrophages, are a major cell type found in ring walls of suppurative granulomas in human listeriosis while T and B cells are virtually absent within these structures. This strongly suggests an inhibitory mechanism that is provided by the cells within the ring wall, thereby prohibiting access of T and B cells to the granulomas. Indeed, by immunohistochemistry, we identified the majority of cells within the ring wall as expressing IDO, an enzyme reportedly associated with strong inhibitory effects on T cell activation (30, 33, 49). IDO was found by transcriptional profiling and quantitative real-time RT-PCR to be the most significantly upregulated gene in human DCs within 4–6 hours after infection with L. monocytogenes. Transcriptional upregulation was followed by a strong upregulation of functional IDO at the protein level as early as 12 hours after infection, which resulted in significant tryptophan depletion and kynurenine accumulation in supernatants of infected DCs. Furthermore, we established that endogenous TNF-α and IFN-γ, which are secreted by the infected DCs, are upstream of IDO induction. Therefore, IDO induction seems to be a strictly cell-autonomous response of DCs as a consequence of L. monocytogenes infection since no other exogenous cells or signals other than infection were necessary. In contrast with the uptake of hk listeria, infection with avirulent mutants of L. monocytogenes also induced IDO expression in DCs, suggesting that the induction of IDO by listeria appears to be independent of currently known virulence factors but requires viable bacteria. Here we could identify LTA as least as 1 Listeria-derived component capable of inducing IDO in DCs. By assessing other infectious diseases and sarcoidosis, a noninfectious granulomatous disease, we seemed to be important host reaction to control pathogens, especially when the latter cannot be eliminated by an effective adaptive immune response. The cellular components and molecular mechanisms involved are strongly dependent on the particular pathogen and the host responsiveness. Most often, macrophages and T cells have been associated with ring-wall formation surrounding such containment structures (1–3). Here we demonstrate that DCs, along with macrophages, are a major cell type found in ring walls of suppurative granulomas in human listeriosis while T and B cells are virtually absent within these structures. This strongly suggests an inhibitory mechanism that is provided by the cells within the ring wall, thereby prohibiting access of T and B cells to the granulomas. Indeed, by immunohistochemistry, we identified the majority of cells within the ring wall as expressing IDO, an enzyme reportedly associated with strong inhibitory effects on T cell activation (30, 33, 49). IDO was found by transcriptional profiling and quantitative real-time RT-PCR to be the most significantly upregulated gene in human DCs within 4–6 hours after infection with L. monocytogenes. Transcriptional upregulation was followed by a strong upregulation of functional IDO at the protein level as early as 12 hours after infection, which resulted in significant tryptophan depletion and kynurenine accumulation in supernatants of infected DCs. Furthermore, we established that endogenous TNF-α and IFN-γ, which are secreted by the infected DCs, are upstream of IDO induction. Therefore, IDO induction seems to be a strictly cell-autonomous response of DCs as a consequence of L. monocytogenes infection since no other exogenous cells or signals other than infection were necessary. In contrast with the uptake of hk listeria, infection with avirulent mutants of L. monocytogenes also induced IDO expression in DCs, suggesting that the induction of IDO by listeria appears to be independent of currently known virulence factors but requires viable bacteria. Here we could identify LTA as least as 1 Listeria-derived component capable of inducing IDO in DCs. By assessing other infectious diseases and sarcoidosis, a noninfectious granulomatous disease, we
demonstrate that IDO+ DCs are associated with granulomatous diseases but absent in nongranulomatous infectious diseases.

Only recently, some of the cells and mechanisms involved in abscess formation by other pathogens, e.g., \textit{Staphylococcus aureus} and \textit{Bacillus fragilis}, were characterized (50). So far, formation of structural containments for bacteria has mainly been attributed to granulocytes, macrophages, and T cells (1, 51, 52). T cells activate macrophages and polymorphonuclear cells, which then adhere to locally activated mesothelium, a requirement for the initiation of abscess formation (50). In acute murine listeriosis, granulocytes and macrophages play a major role in abscess formation in the liver, where DCs were shown to be absent (7). In contrast, we show here in advanced-stage human listeriosis that DCs, together with macrophages, are a major component of the ring-wall structure of granulomas while granulocytes are mainly found in the center of granulomas. This structural contribution itself suggests an unexpected and new function of DCs at the forefront of defense against intracellular pathogens. Differences between murine models of listeriosis and our findings in human listeriosis suggest that this foodborne disease has a rather different pathology in systemically infected mice, probably due to the different routes of infection. With the emergence of new animal models of listeriosis (53), however, it will become possible to study the role of DCs in the pathophysiology of human chronic listeria infection.

The strong enrichment of DCs within the ring wall and the almost complete absence of T cells in this area were striking. We postulated that prohibiting T cells to enter these ring-wall structures would require efficient inhibitory signals. Recent findings clearly demonstrated that tolerogenic or inhibitory DCs express IDO (33, 54, 55). In fact, S100C/CD11c+ DCs and also CD68+ macrophages within the ring-wall structure expressed substantial amounts of this immunoregulatory enzyme (Figure 1A). Interestingly, although in vitro–generated macrophages produced comparable amounts of TNF-α and IFN-γ, they did not induce IDO to as high levels as seen in DCs, both in vivo and in vitro. There has been debate about the role of tryptophan depletion by IDO+ DCs for T cell inhibition since DCs are normally dispersed in rather low frequencies throughout lymphoid tissues, and therefore a significant gradient of tryptophan and an accumulation of its toxic metabolite kynurenine might actually not be apparent (56). However, a dense wall of IDO+ cells surrounding granulomas (Figure 1A) could result in local consumption of tryptophan and accumulation of kynurenine, leading to T cell inhibition in vivo, as has been shown previously by others and by us for human DCs in vitro (33, 40, 55).

The major function of granulomatous structures is the containment of otherwise uncontrollable pathogens. Therefore, destruction of granulomas by cytotoxic T cells would be counterproductive for the survival of the host (1, 57). Clear evidence about the destructive role of T cells on established granulomas stem from experiments in TNF-α−/− mice (58) infected with mycobacteria. These mice quickly die from respiratory failure after destruction of bacteria-induced granulomas. Destruction of granulomas is accompanied by an uncontrolled type 1 immune syndrome characterized by expansion of activated IFN-γ–producing T cells. The immune response leading to granuloma destruction and bacterial dissemination in these mice is prevented by depletion of T cells, which prolongs survival of the animals (58).

As outlined above, the formation and maintenance of granulomas is TNF-α dependent (58–61). However, events downstream of TNF-α signaling leading to the maintenance of granulomas are still not well characterized. In DCs infected with \textit{L. monocytogenes}, significant TNF-α production is a major element of the cellular response. Using different neutralizing TNF-α antibodies, including the clinically used antibody infliximab, we demonstrate that expression and function of IDO after \textit{L. monocytogenes} infection depends on TNF-α expression. This places IDO downstream of TNF-α in \textit{L. monocytogenes} infection. Localized IDO expression by cells within granulomas might be one of the mechanisms keeping T cells at bay. In patients with rheumatoid arthritis treated with TNF-α–neutralizing therapy using infliximab or etanercept (Enbrel), fulminant exacerbation of tuberculosis, listeriosis, and other granulomatous infections has recently been recognized as a severe adverse event (62–65). Interestingly, in patients with Crohn disease, characterized by high local expression of IDO, treatment with infliximab led to reduced IDO expression (66). Hence, inhibition of immunoregulatory mechanisms, such as tryptophan catabolism by IDO, as a consequence of TNF-α–neutralizing therapy are likely to play an important role during disaggregation of preexisting granulomas in tuberculosis and listeriosis.

Taken together, our findings in human listeriosis suggest a new function of DCs. In addition to their important role for the induction of specific immunity, DCs seem to be a major contributor of the cellular components of suppurrative granulomas. Moreover, the expression of the immunoregulatory enzyme IDO by these DCs strongly suggests that not only structural but also metabolic mechanisms are involved in the granulomatous containment of intracellular pathogens, such as \textit{L. monocytogenes} or \textit{B. henselae}, in humans. Our findings also warrant further exploration of the in vivo role of DCs during infection with \textit{L. monocytogenes} and other life-threatening intracellular pathogens, using genetically engineered and clinically relevant animal models, once these become available (53).

**Methods**

**Blood and lymph node samples.** Blood samples were collected from healthy blood donors at the Center for Transfusion Medicine (University of Cologne, Cologne, Germany) after informed written consent was obtained. Lymph node specimens were acquired from the pathology archive of the Institute for Pathology (University of Cologne). For these experiments, approval was obtained from the University of Cologne Institutional Review Board.

**Histochemistry.** Lymph node specimens from 3 patients with clinically and serologically confirmed listeriosis were available from the local pathology archive. Conventional histological staining (H&E, van Gieson, PAS, and Ziehl-Neelsen) was performed according to standard procedures (IHC World; http://www.ihcworld.com) using paraffin-embedded tissue samples. For immunohistochemistry, CD3, CD15, CD20, CD68, and S100 mAbs were obtained from Dako; IDO polyclonal Ab from AbD Serotec; and CD11c from Novocastra. For double-labeling fluorescence microscopy, CD68-FITC was obtained from Dako. Anti–mouse Cy2, anti–sheep Cy3, anti–rabbit Cy2 and anti–rabbit Cy3 were obtained from Jackson and other life-threatening intracellular pathogens, using genetically engineered and clinically relevant animal models, once these become available (53).
Virulence factor expression in L. monocytogenes. Genomic DNA of L. monocytogenes, M. tuberculosis, and B. henselae was identified by PCR. Paraffin wax–embedded lymph node sections were dewaxed in xylene (65°C, 20 min), washed in 100% ethanol, and then lysed in protease K buffer (500 μg/ml proteinase K [Invitrogen]), 50 mM Tris/HCl, pH 7.4, and 5 mM EDTA, pH 8). Nucleic acids were extracted by phenol/chloroform and subsequently precipitated with sodium acetate and isopropanol. Primers used are listed in Supplemental Table 1.

RNA preparation, microarray hybridization, and data processing. DCs were harvested 2, 6, and 24 hours after infection, lysed in TRizol reagent (Invitrogen), and stored at −80°C until further processing. Target preparation and array hybridization (HG-U133A, Affymetrix; and Sentrix Human-6 Expression BeadChip array; Illumina) were performed as described previously (74, 75). All arrays were normalized with dChip 2005 at probe intensity level against the array with overall median signal intensity. The following filtering criteria were used for selection of differentially expressed genes: lower boundary of the 90% confidence interval of the fold change greater than 2, absolute difference in signal intensity between group means greater than 100, and percentage of present calls of probe sets greater than or equal to 50. For data analysis and visualization, R software version 2.2.1 (http://www.r-project.org) (76), GeneSpring 7.2 (Agilent Technologies), and Mayday 2.0 (http://www.zbt.uni-tuebingen.de/psa/mayday) were used (77).

ELISA and EIA. IFN-γ, TNF-α, IFN-β, and PGE2 metabolites in supernatants were measured by human IFN-γ and TNF-α Eli-Pair kits (Diaclone Research), Human Interferon Beta ELISA Kit (R&D Systems), and Prostaglandin E Metabolite EIA Kit (Cayman Chemical), respectively, according to manufacturers’ instructions.

Quantitative real-time PCR. Quantitative real-time PCR was performed with a LightCycler TaqMan Master kit and a Universal ProbeLibrary Assay on a LightCycler 1.3 and analyzed by LightCycler 3 and RelQuant software (version 1.0; Roche Diagnostics) using a calibrator-normalized relative quantification approach. Relative quantification was based on β2 microglobulin expression. Primers used are listed in Supplemental Table 1.

Immunoblot analysis. Immunoblot analysis was performed as previously described (33, 78). The following antibodies were used: monoclonal (Chemicon International) or polyclonal anti-IDO antibody (AbD Serotec); polyclonal anti–COX-2 antibody (IBL); and monoclonal β-actin antibody (Sigma-Aldrich). Recombinant human IDO (rhIDO) served as positive control.

Assessment of tryptophan and kynurenine concentration in supernatants. Supernatants from DC cultures were collected 6, 12, and 24 hours after infection, diluted 1:30 in double-distilled water, and assessed for tryptophan concentrations by HPLC as previously described (33, 49). Tryptophan medium concentration was 5 μg/ml for CellGro DC medium (CellGenix) (as measured by HPLC). Kynurenine was assessed using a spectrophotometric assay as previously described (40, 49). Supernatants were mixed with 30% trichloroacetic acid (2.1%), vortexed, and centrifuged at 8,000 g for 5 minutes. Subsequently, 75 μl of this mixture was added to an equal volume of Ehrlich reagent (100 mg p-dimethylbenzaldehyde, 5 ml glacial acetic acid) in a 96-well microtiter plate. Samples (in triplicate) were run against a standard curve of defined kynurenine concentrations (0–100 μg/ml). Optical density was measured using a Medgenix 400 AT microplate reader (SLT Instruments) at 492 nm within 10 minutes.

Statistics. Statistical significance was calculated by a 2-sample 2-tailed Student’s t test (R software version 2.2.1). Comparisons were considered statistically significant if P < 0.05.

Acknowledgments We are grateful to all blood donors for donating blood for this study. We thank Birgit Gathof from the Center for Transfusion Medicine for providing us with peripheral blood products. We also thank Alexander Poyarkov for helpful discussion during the preparation of this manuscript and Thomas Quast and Waldermar Kolanus for help with confocal microscopy. We thank Nektaria Papadopoulou for preparation of rhIDO and K. Schrör and...


