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A Subset of Human Dendritic Cells in the T Cell Area of Mucosa-Associated Lymphoid Tissue with a High Potential to Produce TNF-α[1]

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Recently, a new class of human dendritic cell (DC) precursors has been described in the peripheral blood recognized by the mAb M-DC8. These cells represent ~1% of PBMC and acquire several characteristics of myeloid DC upon in vitro culture. In this report we show that M-DC8$^+$ monocytes secrete in response to LPS >10 times the amount of TNF-α as M-DC8$^-$ monocytes, but produce significantly less IL-10. Consistent with a role in inflammatory responses, we found that M-DC8$^+$ cells localized in the T cell area of inflamed human tonsils and in the subepithelial dome region of Peyer's patches. In patients with active Crohn's disease, abundant M-DC8$^+$ cells were detectable in inflamed ileal mucosa, which were entirely depleted after systemic steroid treatment. Our results indicate that M-DC8$^+$ cells are cells of DC phenotype in inflamed mucosa-associated lymphoid tissue that may contribute to the high level of TNF-α production in Crohn's disease. We infer that selective elimination of M-DC8$^+$ cells in inflammatory diseases has therapeutic potential. ***The Journal of Immunology,*** **2003, 170: 5089–5094.**

The mAb M-DC8 has been generated by immunization of mice with lineage marker-negative human PBMC (1). The Ab exclusively stains ~30–50% of CD14^{low}/CD16^{++}/CD64$^-$ monocytes that are capable of using CD16 for Ag uptake and are activated by cross-linking of CD16 (2). Similar to the classical CD14^{bright} monocytes, M-DC8$^+$ monocytes develop in the presence of GM-CSF and IL-4 into dendritic cells (DCs)[3] with a high potential to prime naive T cells (2). DCs develop from bone marrow precursors and exist as sentinels in lymphoid and nonlymphoid organs. Langerhans cells in the epidermis and dermal DCs in the dermis are examples for the specialized phenotype of DCs in different localizations of the tissue.

As sentinels, the DCs exhibit an immature phenotype with a particular capacity for Ag uptake and processing. In the mouse, DCs can be specifically detected by CD11c staining, and there is evidence that an inflammatory stimulus can elicit the development of mouse monocytes into lymph node DCs in vivo (3). It is not known whether the same is true for M-DC8$^+$ monocytes in humans, albeit DCs derived from M-DC8$^+$ monocytes in vitro show a superior capacity to prime naive T cells (2). Although the M-DC8 Ag has not yet been identified, its expression seems to be highly specific, is maintained during DC differentiation in vitro, and to date has not been detected on any other cell type (1, 2). Therefore, the M-DC8 Ab may offer the opportunity to trace M-DC8$^+$ cells in human lymphatic tissue.

In this report we show that despite their low frequency in peripheral blood, M-DC8$^+$ cells are a major source of TNF-α in response to bacterial LPS and are significantly expanded in patients with bacterial sepsis. We further identify and characterize M-DC8$^+$ cells in inflamed tonsillar and small intestinal tissue with a morphology described for murine DC. Moreover, we demonstrate that M-DC8$^+$ DCs are located immediately underneath the epithelium in tonsils and Peyer's patches, a localization closest to incoming Ags transported by M cells (4). Based on these observations, M-DC8$^+$ cells appear as a proinflammatory cell type that is recruited to inflamed mucosa-associated lymphatic tissues in response to both bacterial and other stimuli. Therapeutic manipulation of M-DC8$^+$ cells may provide a more selective approach for the treatment of disease states that are thought to be related to excessive TNF-α secretion.

Materials and Methods

Blood and tissue samples

Peripheral blood was obtained from healthy volunteers, and from leftovers of heparinized blood samples that were taken for routine clinical purposes from nine patients with bacterial sepsis severe enough to require intensive care admission. Tonsillar tissue was obtained from patients who were tonsillectomized for recurrent tonsillitis. Only tissue not required for other pathological studies was used. Ileal biopsies were obtained during the initial diagnostic colonoscopy from three patients with recent onset of Crohn's disease, who had not yet received anti-inflammatory treatment. In two patients ileal biopsies were also available following a course of prednisone treatment. All patients were treated according to standard clinical protocols, and all blood and tissue samples were obtained according to the regulations of the local ethical committee and confirm to the Declaration of Helsinki.

Media and reagents

The medium used throughout was RPMI 1640 supplemented with 2 mM L-glutamine, 1% nonessential amino acids, 1% pyruvate, 50 μg/ml kanamycin (Life Technologies, Grand Island, NY), 5×10^{-5} M 2-ME (Merck, Darmstadt, Germany) and 10% FCS (HyClone Laboratories, Logan, UT), hereafter referred to as complete medium. Human rIL-4 was produced in

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[3] Abbreviations used in this paper: DC, dendritic cell; CD40L, CD40 ligand; Dex, dexamethasone; GC, germinal center; PI, propidium iodide; SED, subepithelial dome.

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our laboratory by PCR cloning and expression in a myeloma-based expression system (5). GM-CSF (Leucomax) was purchased from Novartis (Basel, Switzerland). Water-soluble dexamethasone (Dex), polyI:C, and LPS (from *Salmonella abortus* equi) was obtained from Sigma-Aldrich (Deisenhofen, Germany). IL-1β and IFN-γ were purchased from R&D Systems (Minneapolis, MN).

Isolation and culture of M-DC8$^+$ and M-DC8$^-$ monocytes

To isolate M-DC8$^+$ cells, human PBMC from healthy blood donors were incubated in RPMI 1640/1% FCS for 30 min at 4°C with undiluted supernatant of the M-DC8 hybridoma containing ~10 μg/ml Ab. After washing three times with RPMI/25 mM HEPES, the M-DC8$^+$ cells were isolated using rat anti-mouse IgM microbeads (30 min at 4°C) and an LS$^+$ separation column according to the manufacturer's protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). A purity >95% was obtained by a second purification step on an MS$^+$ separation column. More than 90% of M-DC8$^+$ monocytes were thus depleted from PBMC. Remaining monocytes from the same donor were isolated from the M-DC8-depleted PBMC using anti-CD14-conjugated magnetic microbeads according to the manufacturer's protocol (Miltenyi Biotec) to a purity of >99%.

In vitro generation of DCs and quantification of cytokines

To obtain immature DC, M-DC8$^+$ and the M-DC8$^-$ cells were cultured at 3×10^5/ml in RPMI/10% FCS supplemented with 50 ng/ml GM-CSF and 1000 U/ml human rIL-4 for 5–7 days.

To determine cytokine production, M-DC8$^+$ and M-DC8$^-$ monocytes were stimulated either immediately or after 5 days of culture in GM-CSF and IL-4. Cells (2×10^5/100 μl) were stimulated in 96-well, flat-bottom plates in complete medium with 100 ng/ml LPS, 20 μg/ml poly(I:C), 10^4 U/ml IL-1β, 1000 U/ml IFN-γ, or with CD40 ligand (CD40L)-transfected J558L cells (at a 1/10 ratio; gift from Dr. P. Lane, Birmingham, U.K.). Supernatants were collected after 40 h. TNF-α and IL-10 were measured using commercially available ELISA kits (BD PharMingen, San Diego, CA).

Purification of M-DC8$^+$ DC from tonsils

Tonsils obtained from children undergoing tonsillectomy were finely minced, digested with 1 mg/ml collagenase IV (Sigma-Aldrich), and separated by centrifugation over Lymphoprep (LSM, Organon Teknika, Rockville, MD). M-DC8$^+$ DC were enriched with magnetic microbeads as described above.

Flow cytometric analysis

Cells isolated from tonsillar tissue were double stained with supernatant from the M-DC8 hybridoma and one of the following FITC-labeled Abs: anti-HLA class I, anti-HLA-DR, CD1a, CD2, CD4, CD11b, CD11c, CD16, CD19, CD20, CD21, CD40, CD54, CD62, CD80, CD83, and CD86 (all from BD PharMingen). CCR1, and CCR5 were provided by M. Mack (University of Munich, Munich, Germany), and the CCR7 Ab was a gift from Dr. M. Lipp (Max Delbrueck Center for Molecular Medicine, Berlin, Germany). An anti-CCR6 PE-conjugated Ab was purchased from BD PharMingen.

As secondary Abs we used human-adsorbed FITC- or PE-conjugated goat F(ab′)$_2$ anti-mouse IgM (Southern Biotechnology Associates, Birmingham, AL) and FITC-conjugated goat anti-mouse IgG Abs. Stained cells were analyzed by flow cytometry using a FACSCalibur cytometer (BD Biosciences, San Diego, CA) equipped with the CellQuest software (BD Biosciences). Propidium iodide (PI) was used to exclude dead cells.

Determination of Dex sensitivity

Isolated monocytes were incubated with Dex at concentrations between 10^{-6} and 10^{-9} M in complete medium with GM-CSF and IL-4. After 12, 24, 72, and 120 h, viability was determined by flow cytometry measuring annexin V staining and PI uptake.

Immunohistochemical staining of human tissues

From paraffin blocks, 5-μm slides were used for immunohistochemical study. The slides were deparaffinized in xylol, rehydrated, and treated three times for 10 min each time with a target unmasking fluid (Kreatech Diagnostics, Amsterdam, The Netherlands). Endogenous peroxidase was blocked by incubation in 1% hydrogen peroxide for 10 min. The slides were then incubated for 1 h with anti-M-DC8 (Micromet, Munich, Germany), followed by incubation with biotin-conjugated goat anti-mouse IgM (DAKO, Carpenteria, CA) for 30 min. After the addition of the peroxidase-antiperoxidase complex (Vectastain ABC; Vector Laboratories, Burlingame, CA) for 30 min, freshly prepared 3-amino-9-ethylcarbazol (DAKO) was used as chromogen. For double stainings the slides were then incubated with with anti-CD1a, anti-CD8, or anti-CD68 (DAKO) for 1 h. For detection of second Abs, a supersensitive alkaline phosphatase/anti-alkaline phosphatase (kit was used according to the manufacturer's recommendations (DAKO code K5000)). Between all steps, the slides were washed twice for 5 min each time in Tris buffer at pH 7.4. The sections were counterstained with Meyer's hematoxylin and mounted with Paramount medium (DAKO).

Statistical analysis

Statistical analysis was performed using the Wilcoxon matched pairs and Mann-Whitney tests. A value of $p < 0.05$ was considered significant.

Results

M-DC8$^+$ cells are major producers of TNF-α

M-DC8 was described as a surface marker of a population of peripheral blood monocytes with a potential to develop into DCs in vitro (2). We have recently compared the functional capacity of M-DC8$^+$ monocytes with M-DC8$^+$ DC differentiated in vitro by addition of GM-CSF and IL-4 (2). Here we extend this comparison to investigate whether M-DC8-positive and -negative monocyte subsets differ with respect to their secretion of the proinflammatory cytokine TNF-α and the anti-inflammatory cytokine IL-10 in response to different activation stimuli. Upon stimulation with LPS, both freshly isolated as well as cultured M-DC8$^+$ DC secreted large amounts of TNF-α (Fig. 1*A*). Freshly isolated peripheral blood M-DC8$^+$ monocytes secreted ~10–14 times higher levels of TNF-α than M-DC8$^-$ monocytes. In vitro differentiation of M-DC8$^+$ monocytes into DCs according to a standard protocol (6) further increased their ability to produce TNF-α by a factor of 2. This was still 4 times more than the TNF-α produced by cultured M-DC8-negative DCs. A contrary picture was obtained with the anti-inflammatory cytokine IL-10 (Fig. 1*B*). Both M-DC8$^-$ monocytes and DCs were producing 5- to 10-fold higher levels of IL-10 compared with M-DC8$^+$ cells. IL-6 production following stimulation with LPS was comparable in M-DC8$^-$ and M-DC8$^+$ cells (data not shown).

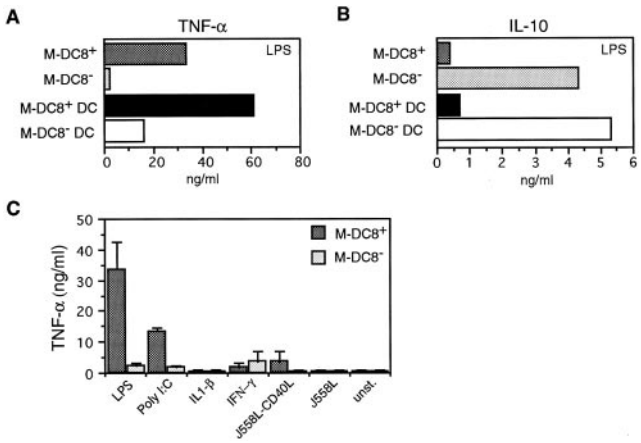

FIGURE 1. TNF-α (*A*) and IL-10 (*B*) production of M-DC8$^+$ and M-DC8$^-$ cells. Following stimulation with LPS, freshly isolated as well as cultured (GM-CSF and IL-4) M-DC8$^+$ cells produce high amounts of TNF-α, but little IL-10 compared with M-DC8$^-$ cells from the same donor ($p < 0.01$, by Mann-Whitney test). Stimulation was performed with either 100 ng/ml LPS for freshly isolated cells or 1 μg/ml for DCs. Cytokines were measured after 40 h of stimulation. *C*, LPS was the strongest stimulus for TNF-α production by M-DC8$^+$ cells, followed by polyI:C. Much lower levels of TNF-α production were elicited by IL-1β, IFN-γ, and CD40L. Data represent the mean values of five different donors.

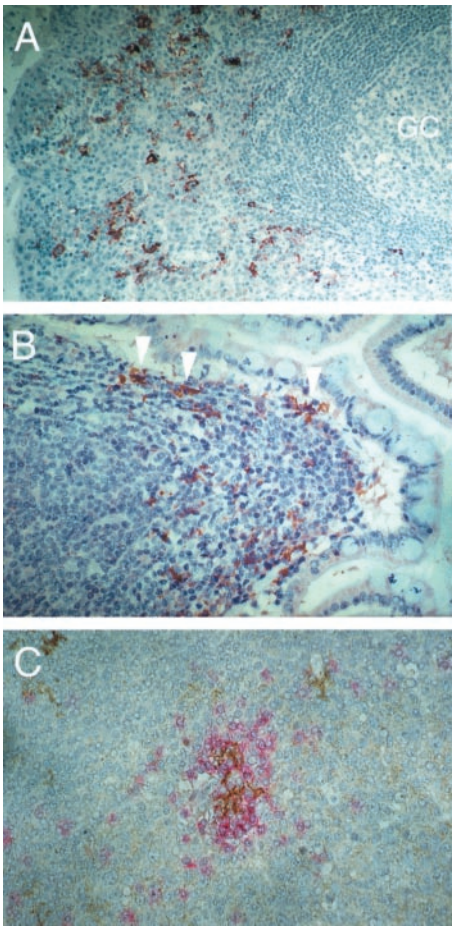

FIGURE 2. Phenotype of M-DC8$^+$ cells in bacterially infected tonsils and reactive Peyer's patches of the ileum. M-DC8 is expressed on cells in regions of the tonsil to which interdigitating DCs are restricted. The sections were counterstained with hematoxylin. *A*, M-DC8 is expressed on interdigitating cells close to the crypt epithelium in human tonsils (magnification, ×200). *B*, M-DC8 single staining on an ileum cross-section (magnification, ×300). Arrows indicate cells with typical DC morphology, i.e., with long interdigitating processes, in Peyer's patches. *C*, Double staining of M-DC8 and CD8 on a tonsil section shows colocalization of both cell types (magnification, ×300).

Various stimuli were compared for their ability to induce TNF-α production in M-DC8-positive and -negative monocytes. The strongest stimulus for the high level TNF-α production by M-DC8$^+$ cells was LPS, followed by polyI:C (Fig. 1*C*). IL-1β, IFN-γ, and CD40L elicited much lower levels of TNF-α production. M-DC8-negative monocytes were only superior in TNF-α secretion after stimulation with IFN-γ.

To test the overall contribution of M-DC8$^+$ cells to LPS-induced TNF-α secretion by PBMC, we depleted PBMC of six healthy donors of M-DC8$^+$ cells using magnetic beads. TNF-α secretion was reduced by a mean of 43 ± 11.5%, which corresponds well to the frequency of M-DC8$^+$ cells (mean, 0.86 ± 0.43% of PBMC) and CD14^{++} monocytes (8.8 ± 3.9%), and the differences in TNF-α secretion seen with the isolated cell populations (see Fig. 1, *A* and *C*). In summary, these data show that M-DC8-positive monocytes and DCs have a more pronounced pro-inflammatory phenotype than monocytes lacking this marker. Moreover, M-DC8-positive monocytes, despite their low frequency in peripheral blood, seem to play a major role in the TNF-α response of PBMC to bacterial LPS.

Table I. *Phenotype of M-DC8$^+$ cells isolated from human tonsillar tissue*

Marker	% Positive Cells	MFI
HLA class I	100	553
HLA-DR	50	196
CD1a	3	6
CD2	76	61
CD4	41	39
CD11c	25	41
CD11b	3	5
CD14	3	21
CD16	2	9
CD40	20	42
CD45RA	38	131
CD45RO	49	237
CD54	87	202
CD62	15	13
CD80	1	3
CD83	6	8
CD86	10	7
CCR1	23	15
CCR5	57	39
CCR6	33	65
CCR7	87	74

The mAb M-DC8 detects DCs in the T cell area of inflamed mucosa-associated lymphoid tissue

M-DC8$^+$ cells have been characterized as a subpopulation of CD14^{low}/CD16^{++} peripheral blood monocytes (2). The latter have been suggested to represent precursors of tissue macrophages (7). We have used the M-DC8 Ab to follow the trafficking of M-DC8$^+$ monocytes in vivo. To this end, we stained paraffin-embedded tissue sections of human palatine tonsil, lymph nodes, spleen, and small intestine with Peyer's patches using the mAb M-DC8. In inflamed tonsils and Peyer's patches of the ileum we detected M-DC8$^+$ cells that exhibited complex cytoplasmic projections typical of DC and very similar to those described for interdigitating DC or Langerhans cells (8) (Fig. 2, *A* and *B*). In noninflamed tissue of lymph nodes or spleen, M-DC8$^+$ cells were rarely detected, and few were found in inflamed lymph nodes (data not shown). In tonsillar tissue, M-DC8$^+$ cells were predominantly localized in the T cell zone and in or next to the nonkeratinizing squamous epithelium of the tonsillar crypts. In Peyer's patches, M-DC8$^+$ cells were detected in the subepithelial dome (SED) region (Fig. 2*B*).

To further characterize M-DC8$^+$ cells and their interaction with other cells in inflamed tonsillar tissue we performed double stainings with M-DC8 and CD8, CD1a, and CD68 on tissue sections. Double staining with CD8 occasionally revealed direct interaction

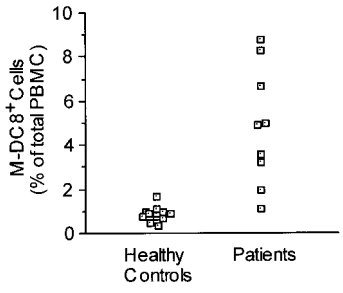

FIGURE 3. Frequency of M-DC8$^+$ cells in the peripheral blood of healthy donors and patients with bacterial infections. Results are expressed as a percentage of the total PBMC. A value of $p < 0.0005$, by Mann-Whitney test.

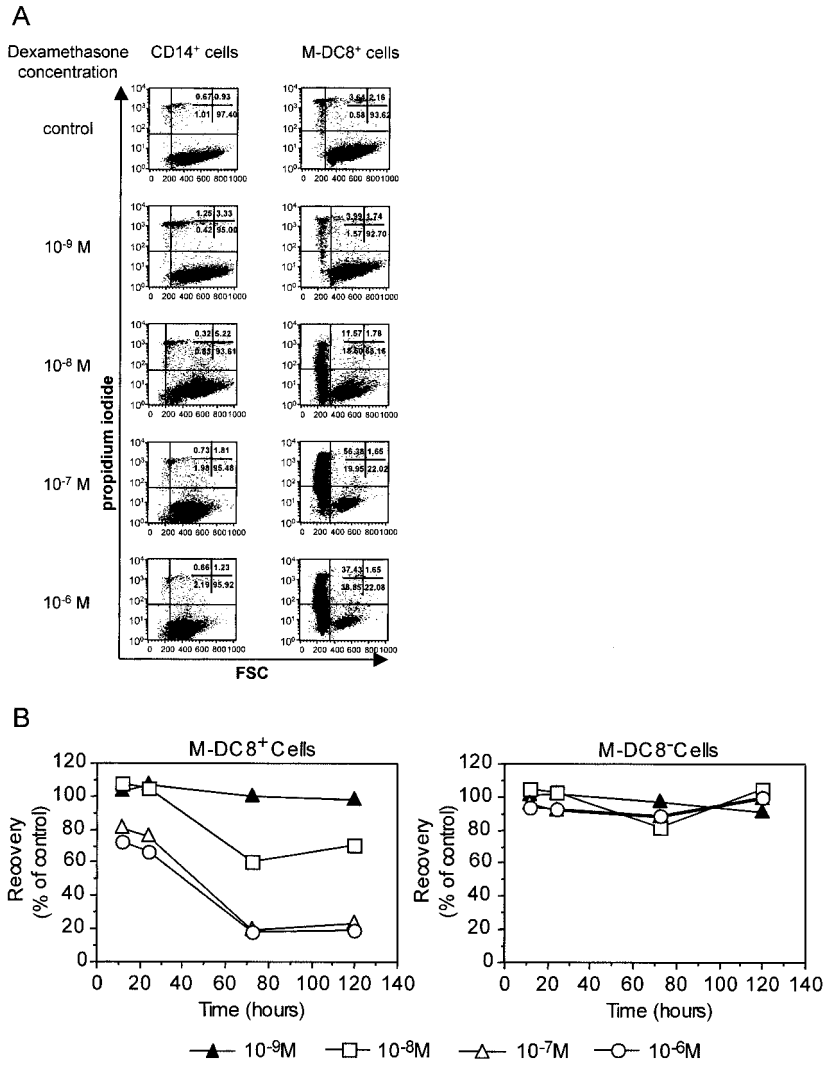

FIGURE 4. The effect of Dex on the survival of freshly isolated M-DC8$^+$ and M-DC8$^-$ cells. Freshly isolated M-DC8$^+$ and M-DC8$^-$ monocytes were cultured for 12, 24, 72, and 120 h in the presence of varying concentrations of dexamethasone. *A*, Flow cytometric analysis of PI uptake of M-DC8$^+$ and M-DC8$^-$ monocytes at 120 h (shown for all different time points in *B*). The data are representative of three independent experiments.

between CD8$^+$ lymphocytes and M-DC8$^+$ cells (Fig. 2*C*). A minority of M-DC8$^+$ cells was positive for CD68; compared with M-DC8 single-positive cells, these cells appeared less veiled (data not shown). M-DC8$^+$ cells were isolated from tonsils and stained for FACS analysis with Abs against DC, macrophage, B and T cell markers, and chemokine receptors. M-DC8$^+$ cells from tonsils were positive for HLA classes I and II, CD4, CD11c, and CD54, but only some of the cells were positive for CD40, and CD62 (Table I). In contrast to peripheral blood M-DC8$^+$ cells, CD16 was absent from M-DC8$^+$ cells in tonsillar tissue, which is in line with our previous observations that peripheral blood M-DC8$^+$ cells down-regulate CD16 upon in vitro differentiation into DC (2). Tonsillar M-DC8$^+$ cells were also negative for CD1a, CD19, CD20, CD80, CD83, and CD86.

All M-DC8$^+$ cells from the tonsil were positive for CCR5 and CCR7, and, dependent on the inflammatory state of the tissue, ~50% were also positive for CCR6. CCR1 was only weakly expressed. Thus, the M-DC8 Ab identifies a population of human myeloid cells with DC morphology that are localized subepithelially in mucosa-associated lymphoid tissue. In contrast to interdigitating DC or Langerhans cells, M-DC8$^+$ cells are negative for CD1a and, despite their immature phenotype with the low expression of costimulatory molecules, seem to interact with CD8$^+$ T cells.

Increased frequency of peripheral blood M-DC8$^+$ cells in patients with bacterial sepsis

M-DC8$^+$ cells are among the strongest TNF-α secretors in peripheral blood in response to LPS and are found with a high frequency in inflamed lymphatic tissues. We therefore asked whether the frequency of peripheral blood M-DC8$^+$ monocytes is changed in patients with bacterial sepsis, where TNF-α is thought to play a major role. Monocytes were isolated from leftovers of heparinized blood samples that were taken for clinical routine from nine patients with severe bacterial sepsis (Fig. 3). We observed a statistically significant higher percentage of M-DC8$^+$ cells in the peripheral blood compared with 11 healthy blood donors (4.7 ± 2.8 vs $0.86 \pm 0.43\%$; $p < 0.0005$). These data suggest that M-DC8$^+$ cells significantly contribute to the high TNF-α production during bacterial sepsis.

Glucocorticoids induce apoptosis in M-DC8$^+$ monocytes at low concentrations

Given the association of M-DC8$^+$ cells with inflammatory conditions, we tested the sensitivity of M-DC8$^+$ cells to Dex, one of the most frequently used immunosuppressive steroid hormone analogs. Dex concentrations between 10^{-9} and 10^{-6} M were tested. Since M-DC8$^+$ monocytes do not survive beyond 48 h in vitro

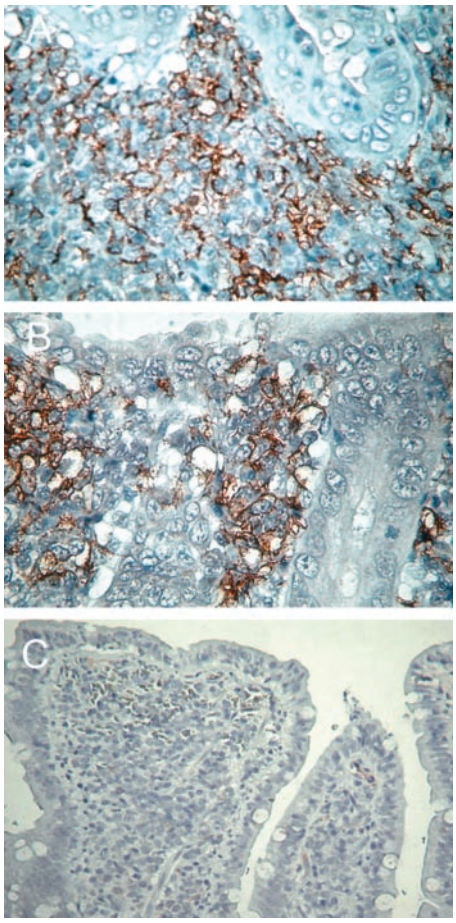

FIGURE 5. Infiltration of M-DC8$^+$ cells in the subepithelial region of the ileum in patients with Crohn's disease. *A* and *B,* Paraffin sections from ileal biopsies from two patients with initial onset of Crohn's disease were stained with the M-DC8 Ab (magnification, ×800). *C,* M-DC8 staining of an ileal biopsy taken 6 mo after corticosteroid treatment (magnification, ×300).

without the addition of growth factors, the experiments were performed in the presence of GM-CSF and IL-4. Dex at concentrations of 10^{-8} M and higher, which correspond to therapeutic in vivo concentrations, induced apoptosis, as determined by PI incorporation in 50–85% of M-DC8$^+$ cells after 3 days (Fig. 4, *A* and *B*). In contrast, the viability of M-DC8$^-$ monocytes was not significantly affected by any of the Dex concentrations tested up to 5 days of culture. The pharmacological dose of Dex ranges between 10^{-8} and 10^{-6} M. This puts the germinal center (GC) sensitivity of M-DC8$^+$ cells in a meaningful range and suggests that induction of apoptosis in M-DC8$^+$ cells may contribute to GC-mediated immunosuppression.

M-DC8$^+$ cells are abundant in the ileum of patients with Crohn's disease and are depleted by glucocorticoid treatment

Crohn's disease is an idiopathic chronic inflammatory bowel disease thought to be autoimmune mediated and is frequently responsive to GC treatment (9). In three patients with recent onset of Crohn's disease, who had not yet received anti-inflammatory treatment, ileal biopsies showed dense infiltration of M-DC8-positive cells in the subepithelial region (Fig. 5, *A* and *B*). Again, a DC-like morphology was apparent. In one patient, a second biopsy was available 6 mo after GC-induced clinical remission. In this biopsy M-DC8$^+$ cells had completely disappeared from the ileal mucosa (Fig. 5*C*).

Discussion

We have shown that the M-DC8 Ab defines in humans a population of very potent TNF-α-producing monocytes in peripheral blood and a class of myeloid cells with DC morphology in mucosa-associated lymphoid tissue.

It has long been known that monocytes produce high levels of TNF-α in response to LPS. However, considering the various monocyte subpopulations, the relative contributions of subpopulations to TNF-α production are unclear. Very recently, it has been shown that CD14$^+$/CD16$^+$/HLA-DR^{++} monocytes are a major source of TNF-α in response to LPS or a bacterial lipopeptide (10). M-DC8$^+$ monocytes in the peripheral blood of healthy individuals represent 40% of the CD14/CD16 double-positive monocytes and 10% of the total circulating monocytes. The M-DC8 Ab allows for the first time the isolation and analysis of this monocyte subpopulation without employing an anti-CD16-Ab, which by itself leads to strong activation (2). Our study shows that bacterial stimulation is able to induce, on the average, a >10-fold higher secretion of TNF-α in M-DC8$^+$ cells compared with M-DC8$^-$ monocytes and that within the CD14/CD16 double-positive subset, the M-DC8$^+$ monocytes are responsible for enhanced TNF-α production. Also, upon LPS stimulation, M-DC8$^+$ monocytes and M-DC8$^+$ DC produce very little IL-10, confirming their strong proinflammatory potential. These findings are also in line with a very recent report by Schakel et al. (11), who showed that the M-DC8 Ab binds to a distinct PSGL-1 variant and that M-DC8$^+$ monocytes express the anaphylatoxin receptors C5aR and C3aR, indicating that these cells can be rapidly recruited to inflammatory sites.

Since M-DC8$^+$ monocytes seem to be the most potent TNF-α-producing cell population in the peripheral blood, we studied these cells in patients with diseases in which TNF-α is believed to play an important role. TNF-α in modest concentrations contributes to protective inflammatory responses and plays a leading role in the response to Gram-negative bacteria (12). We showed that the frequency of M-DC8$^+$ monocytes in the peripheral blood significantly increases during severe systemic bacterial infection, suggesting that M-DC8$^+$ cells are involved in the antibacterial immune defense, but may also be involved in the detrimental role of TNF-α during septic shock.

In tissue sections we found M-DC8$^+$ cells with a DC morphology in the T cell area and especially in close vicinity to the crypts of inflamed tonsils. Although in humans there is no pan-DC marker like the murine CD11c, the phenotype of the M-DC8$^+$ cells (HLA-DR^{++}/CD11c$^+$/CD45RA$^+$/CD45RO^{++}/CD2$^+$) matches exactly a subset of human tonsillar DC described by Summers et al. (13). In Peyer's patches, M-DC8$^+$ cells were found in the SED region. The surface epithelium over these domes contains M cells that are able to transcytose intact antigenic macromolecules from the lumen to the underlying lymphocytes, thus serving as an important afferent limb in the intestinal immune system (4). Thus, M-DC8$^+$ cells in the SED of the Peyer's patch might be an important cell type for processing and presentation of the intestinal Ag delivered by M cells. The location of M-DC8$^+$ cells in the Peyer's patch, their immature phenotype, and the expression of CCR6 and CCR7 show many similarities to the SED-DCs described in the mouse (14–16).

GCs are potent anti-inflammatory and immunosuppressive drugs. Their therapeutic effects are assumed to be due to their strong inhibitory effect on T cells and the down-regulation of proinflammatory cytokine production in monocytes and macrophages (17, 18). Previously, it has also been demonstrated that GC induce apoptosis in human monocytes (19). In one GC-treated patient, we observed very low levels of M-DC8$^+$ cells, while CD14^{++} monocyte quantities were even increased under steroid treatment (data not shown). This

observation is consistent with our in vitro studies showing that M-DC8$^+$, but not M-DC8$^-$, monocytes are exquisitely sensitive to GC-induced apoptosis. Based on these data we hypothesize that depletion of M-DC8$^+$ cells by GC could represent a new mechanism of GC-induced immunosuppression.

Given the high potential for TNF-α secretion, their subepithelial localization in small intestinal mucosa, and their sensitivity to GC, it is tempting to speculate that M-DC8$^+$ cells could be involved in the pathogenesis of inflammatory bowel disease, especially Crohn's disease. High levels of TNF-α have been demonstrated in inflamed intestinal mucosa in Crohn's disease (20, 21), and strong evidence for an important pathogenetic role of TNF-α comes from the successful clinical use of anti-TNF-α Abs in GC refractory disease (22, 23).

In three patients with untreated Crohn's disease we found a high number of M-DC8$^+$ cells in the subepithelial region, and GC treatment resulted in complete depletion of M-DC8$^+$ cells from the mucosa. Further functional characterization of M-DC8$^+$ cells should clarify whether local TNF-α secretion of M-DC8$^+$ cells promotes tissue damage in Crohn's disease and whether depletion of M-DC8$^+$ cells by GC is one mechanism of steroid responsiveness in Crohn's disease. Since it has been suggested that anti-TNF-α Abs mediate their action not only by neutralizing soluble TNF-α, but also by depleting TNF-α-secreting cells, it would be interesting to know whether anti-TNF-α Abs would also deplete M-DC8$^+$ cells (24), making M-DC8$^+$ cells a promising new target for immunosuppressive therapy in Crohn's disease.

In conclusion, we identified M-DC8$^+$ monocytes as the major TNF-α-producing cell in the peripheral blood. In inflamed mucosa-associated lymphoid tissue, M-DC8$^+$ cells with DC phenotype have been found that may play an important proinflammatory role in infectious as well as autoimmune disease and are exquisitely sensitive to treatment with glucocorticoids.

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