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IL-10 Acts As a Developmental Switch Guiding Monocyte Differentiation to Macrophages during a Murine Peritoneal Infection

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The peritoneal wash of BALB/c or C57BL/6 mice contains two populations of macrophages that differ in their level of expression of MHC class II (MHC II). Although both populations efficiently phagocytose bacteria in vivo, only the MHC II<sup>lo</sup> population is effective at phagocytosing apoptotic cells in vivo and only the MHC II<sup>hi</sup> population is effective at presenting Ag to T cells in vitro. Soon after induction of a peritoneal infection both of these macrophage populations are lost from the peritoneal wash fraction. Blood monocytes then enter the inflamed peritoneum and develop into new peritoneal macrophages. Whether these monocytes develop into MHC II<sup>lo</sup> or into MHC II<sup>hi</sup> macrophages is crucially dependent on the cytokine IL-10, which is transiently elevated in the peritoneal wash during the early phase of infection. Monocytes from CD45.1 animals transferred early in infection when the IL-10 concentration is high into congenic CD45.2 recipients develop into the MHC II<sup>lo</sup> macrophage population. Monocytes transferred later, when the IL-10 concentration has fallen, develop into the MHC II<sup>hi</sup> population. In infected IL-10–deficient animals monocytes fail to develop into the MHC II<sup>lo</sup> population but can be induced to do so by exogenous application of IL-10. Finally, high numbers of wild-type monocytes injected into IL-10R1-deficient animals develop into MHC II<sup>lo</sup> macrophages and were able by a bystander effect to induce the differentiation of the endogenous monocytes to the same fate. The Journal of Immunology, 2012, 189: 000–000.

M any strikingly different macrophage types are found in peripheral tissues. Despite this large variety of forms and functions, most, perhaps even all, of these diverse macrophage populations develop in situ from circulating blood monocytes. Monocytes are produced from a precursor stem cell in the bone marrow (1) and then enter the circulation where they remain with a dwell time of ~22 h prior to their extravasation into peripheral tissues (2). There are two well-characterized populations of circulating monocytes that differ in the molecules they express on the surface and in their functional properties (3, 4). However, it is not thought likely that each different macrophage population derives from a separate monocytic line but rather that tissue-specific cues direct monocytes to differentiate into the different macrophage forms. What these cues may be and how they may direct monocyte differentiation in vivo are largely unexplored (5).

Even within one tissue quite different macrophage functions are required at different times. An inflammatory disturbance of tissue homeostasis caused by infection requires a finely orchestrated response (6). Initially macrophages with sentinel function will be needed to detect and signal an incipient infection. This typically results in the recruitment of large numbers of neutrophils, which avidly phagocytose bacteria. The neutrophils rapidly die (7) and must be removed by macrophages, which are efficient at phagocytosing apoptotic cells. Thereafter, any bacteria that have survived this phagocytic response must be dealt with by the adaptive immune system, and this requires populations of macrophages able to act as APCs (8). Finally, during the recovery phase macrophages with an “alternatively activated” phenotype will be required to aid in tissue remodeling and angiogenesis (9). This suggests that tissue-specific and danger-specific cues will play important roles in defining the developmental fates of monocytes in peripheral tissues.

The peritoneum of the mouse is readily accessible and has been particularly well suited to studying cell dynamics during infection. Peritonitis usually results from kidney or liver failure or from a perforation of the gastrointestinal tract, and we have modeled this latter situation by injecting bacteria collected from the cecum into the peritoneum of a recipient. The uninfected peritoneum lacks neutrophils but early in the course of a peritoneal infection (10) many millions of these cells flood into the peritoneal cavity (11). We show that the IL-10, copiously but transiently produced during the initial phase of a polymicrobial peritonitis, plays a decisive role in directing the choice of monocyte developmental fate in the infected mouse peritoneum.

Materials and Methods

Mice

BALB/c, C57BL/6, and C57BL/6 CD45.1 mice were purchased from Charles River. BALB/c CD45.1 animals were a gift from Prof. Lars Nitschke (University of Erlangen-Nurnberg, Erlangen, Germany). BALB/c IL-10–deficient mice were a gift from Prof. Alf Hamann (Charité, Berlin, Germany). DO11.10 mice were a gift from Dr. Van Trung Chu (Deutsches Rheuma-Forschungszentrum, Berlin, Germany). The IL-10-R1 deficient mice (12) were bred and maintained in the Animal Facility at the Uni-
IL-10 DIRECTS MONOCYTE FATE IN INFLAMMATION

Infection with cecal contents

The cecum of a mouse was removed and its contents were weighed and resuspended in 1 ml PBS/100 mg cecal contents (CC). The suspension was filtered through a 70-μm nylon cell strainer. BALB/c mice were injected i.p. with a sublethal dose, which was an amount equivalent to 15 mg CC. Because mice deficient in IL-10 or the IL-10R1 are highly susceptible to peritonitis, experiments that included these animals were conducted using 2 mg CC (13, 14). In some experiments 0.5% 4% Brewer thiglycollate medium was used to induce a sterile peritoneal inflammation.

Cell preparations

For blood monocytes, PBMCs were prepared from whole blood by density gradient centrifugation on Histopaque 1083. The PBMCs were negatively magnetically sorted using Abs to CD11b, F4/80, CD19, B220, CD1c, CD90, DX5, MHC II, SiglecF, and TER119 magnetic beads. The cells were labeled with CellTracker Violet and anti-TER119. The isolated CD4+ cells were labeled with 2.5 mg anti-CD3e, CD19, SiglecF, CFSE in 1 ml prewarmed PBS at 37˚C for 5 min (16).

For peritoneal macrophages, peritoneal wash cells were harvested and negatively selected by magnetic sorting using Abs to CD3e, CD19, SiglecF, TER119, DX5, and, in the case of preparations from infected animals, Ly6G.

For thymocytes, the cell suspension prepared from a mechanically disrupted thymus of a 4-wk-old BALB/c CD45.1 mouse was passaged through a 70-μm nylon cell strainer and then through a 30-μm nylon cell strainer. For T cell priming experiments the cells from axillary and mesenteric LNs were negatively selected using magnetic microbeads nylon cell strainer. BALB/c mice were injected i.p. with a sublethal dose, which was an amount equivalent to 15 mg CC 3 d previously. Peritoneal wash cells were cultured in 0.5 ml RPMI 1640 plus 10% FCS in the presence of 10 μg BrdU for 24 h. BrdU incorporation assay was assayed using the FITC-BrdU flow kit from BD Biosciences according to the manufacturer’s protocol. As a high control, 1 × 106 mesenteric LN cells from DO1.10 mice were cultured in 0.5 ml RPMI 1640 plus 10% FCS in the presence of 10 μg BrdU; 100 ng OVA, and 100 ng LPS for 4 d The cells were then harvested, stained with appropriate Abs, and analyzed by FACS. Assays were done in triplicate.

IL-10 determination

IL-10 in peritoneal washes was determined using a solid phase cytometry-based assay kit (Bender MedSystems) according to the manufacturer’s protocol.

Statistical analyses

The p values for the differences between groups were determined using a Student t test.

Results

Macrophage populations in the normal peritoneum

The macrophage populations present in the peritoneal wash fraction of normal healthy BALB/c mice were labeled with Ab to the myeloid marker CD11b. Two distinct populations of CD11b+ cells are apparent that differ in their expression of MHC class II and F4/80 (Fig. 1A). The minor population is CD11bhi macrophages expresses high levels of MHC II and low levels of F4/80. We refer to these cells hereafter as the MHC IIhi macrophage population. The majority population is CD11bm, MHC class IIlo, and F4/80hi and is referred to hereafter as the MHC IIlo macrophage population. Cells of both populations have the typical morphology of mononuclear phagocytes with a kidney-shaped nucleus and large vacuoles (Fig. 1B). However, the MHC IIlo cells are larger and have higher side scatter and higher autofluorescence than do the MHC IIhi cells (Fig. 1C, 1D). Neither of these cell populations detectably expresses the Ly6G marker of neutrophils, the eosinophil marker SiglecF, the monocyte marker Ly6C, the B cell marker CD19, the T cell marker CD3ε, the NK cell marker DX5, or the mast cell marker c-KIt. The MHC IIlo population expresses CD11c weakly whereas both populations express the costimulatory molecule CD80 (Fig. 1D).

Functional properties of the two peritoneal macrophage populations

Ag presentation to T cells and the ability to phagocytose apoptotic cells are two major roles that tissue macrophages may play. We tested the capacity of the two peritoneal wash macrophage populations to carry out these functions.

For test to Ag presentation capacity, peritoneal macrophages were prepared from BALB/c CD45.1 mice, labeled with fluorochrome-coupled Abs to CD11b, CD4/80, MHC II, and Gr1, and the
MHC IIhi and MHC IIlo populations were then isolated by FACS sorting. CD4+ T cells were prepared from BALB/c DO11.10 CD45.2 mice, which carry a transgenic TCR specific for the chicken OVA peptide 322–339. These T cells were labeled with CFSE and stimulated in vitro with the OVA peptide presented by each of the two macrophage populations (16). The MHC IIhi peritoneal macrophages efficiently presented this peptide and after 4 d 38.3% (n = 3, SD = 1.1%) of the T cells had proliferated. Under the same conditions the MHC IIlo cells did not detectably promote T cell proliferation (Fig. 2A).

To test the capacity of these two macrophage populations to phagocytose apoptotic cells, we prepared thymocytes from BALB/c CD45.2 mice, labeled them with the CellTracker dye BMQC, and then induced apoptosis with dexamethasone. The labeled cells were injected into the peritoneal cavity of BALB/c CD45.2 animals along with 15 mg CC into the peritoneum of CD45.1 mice, labeled them with the CellTracker dye BMQC, and then induced apoptosis with dexamethasone. The labeled cells were injected into the peritoneal cavity of BALB/c CD45.2 animals and 1 h later peritoneal wash cells were recovered and assayed for BMQC-labeled macrophages that were CD45.2+ but CD45.1−. By this assay the MHC IIlo peritoneal macrophages were able to engulf the apoptotic targets whereas the MHC IIhi cells were, by comparison, inefficient at doing so. By measuring the mean fluorescence index of the BMQC-labeled fraction of each population we show that the MHC IIlo population is not only more extensively labeled but that on a per cell basis these cells have taken up substantially more of the apoptotic thymocytes than have the MHC IIhi macrophages (Fig. 2B). Nevertheless, although the MHC IIlo population is markedly more effective at phagocytosing apoptotic cells, both populations are well able to phagocytose bacteria (Fig. 2C).

**Turnover of the peritoneal MHC IIhi and MHC IIlo populations in the steady-state**

To determine the stability of the MHC IIhi and MHC IIlo populations in the mouse peritoneum, total peritoneal wash cells prepared from BALB/c CD45.2 animals were transferred into the peritoneum of CD45.1 congenic mice. The fate of these transferred cells was followed during a period of 7 d (Fig. 3). The MHC IIhi population turns over with a 1/2 of ~3 d, whereas, in contrast, the MHC IIlo population has a considerably longer turnover time.

**Changes in peritoneal wash cell population on infection**

Bolus injection i.p. of CC equivalent to 15 mg cecal wet weight gives rise to a peritonitis characterized in the initial phase by a rapid influx of neutrophils that destroy many of the bacteria. Tracking the kinetics of bacterial clearance is complicated by the fact that the cecal population is largely composed of anaerobes, many of which cannot be cultured. We therefore used the minor population of aerobes as a biological tracer to follow bacterial fate. The initial 5 × 10⁶ CFU aerobes in the bolus is reduced in 24 h to 5 × 10⁴ CFU and then slowly recovers to a value of 1 × 10⁵ CFU 3 d postinfection.

We next examined the macrophage populations in the peritoneal wash fraction 1 h and 3 d postinfection (Fig. 4A). Both the MHC IIhi and the MHC IIlo populations are lost from the peritoneal wash fraction during the first hour of infection, but by 3 d both populations are re-established and reach levels in excess of those present prior to infection (Fig. 4B). The re-established macrophage populations were assayed for their Ag-presenting and phagocytosis properties, and in these respects they do not differ from the MHC IIhi and the MHC IIlo populations present prior to infection, although the cells in the two populations show no size difference and both now have a mean diameter of 20 μm (data not shown).

To determine whether the macrophage populations that are lost in the first hours of infection return to the peritoneum later, we transferred 1 × 10⁶ total peritoneal wash cells from CD45.2 animals along with 15 mg CC into the peritoneum of CD45.1 recipients. The infection causes both the transferred MHC IIhi and the MHC IIlo macrophage populations to disappear from the peritoneum and at day 3 <5% of the transferred peritoneal macrophages were recovered (Fig. 4C). This indicates that the macrophage populations that are re-established in the peritoneum 3 d postinfection do not derive to any significant extent from cells that were present in the peritoneum at the start of the infection.

**Monocyte transfers**

To determine whether the peritoneal macrophage populations formed postinfection derive from blood monocytes, we transferred monocytes from BALB/c CD45.1 mice into CD45.2 recipients (Fig. 5A). Monocytes transferred i.v. into untreated animals do not detectably enter the peritoneum (4, 18) although they do enter the inflamed peritoneum, albeit with low efficiency. To be able to see entry into the peritoneum after i.v. transfer, we treated BALB/c CD45.2 animals with thioglycollate to induce a sterile peritoneal inflammation and then transferred 1 × 10⁷ CD45.1 monocytes either i.p. or i.v. The fate of the transferred monocytes entering the
Recovery of the MHC IIhi macrophage population.

BMQC+MHC IIhi and of the BMQC+MHC IIlo populations (mean basis was compared from the mean fluorescence intensity (MFI) of the stained with Ab to CD45.2 and appropriate Abs. (TruCount beads were added to aliquots that were peritoneal wash cells were recovered by lavage.

BALB/c CD45.1 recipients. One, 3, or 7 d later the peritoneal wash cells were recovered by lavage. The fraction of CD45.1+CD45.2+BMQC+ cells was determined. Right panel. The relative apoptotic cell uptake on a per macrophage basis was compared from the mean fluorescence intensity (MFI) of the BMQC+MHC IIhi and of the BMQC+MHC IIlo populations (mean ± SD, n = 4). ***p < 0.001 by Student t test. (C) The experiment was repeated using FITC-labeled E. coli in place of apoptotic cells (mean ± SD, n = 4). **p < 0.01, ***p < 0.001 by Student t test.

The results show that the conversion of the monocytes into the MHC IIlo type (Fig. 6A), whereas in the infected animals they are of the MHC IIhi type (Fig. 5C). We conclude that the development of the macrophages within the peritoneum is independent of whether transfer is by the i.p. or the i.v. route.

To compare monocyte development in infected and in noninfected mice we transferred BALB/c CD45.1 monocytes i.p. into BALB/c CD45.2 animals. The recipients were either untreated or were infected with CC at the time of monocyte transfer. The results show that the CD45.1.1 peritoneal macrophages recovered on day 3 from noninfected animals are of the MHC IIhi type (Fig. 6A), in the infected animals they are of the MHC IIlo type (Fig. 6B). Infection thus radically alters the populations of macrophages recovered in the peritoneal wash.

**FIGURE 2.** Functional characterization of the MHC IIhi and MHC IIlo peritoneal macrophages. (A) CFSE-labeled CD4+ T cells isolated from the LNs of BALB/c DO11.10 CD45.2 mice were cocultured with FACS-sorted MHC IIlo or MHC IIhi peritoneal wash macrophage populations at a ratio of 5:1 (T cells/macrophages) in the presence of 20 ng OVA peptide and 20 ng LPS for 4 d. Cells were analyzed by FACS after gating on the CD45.2 population. The data are representative of two similar independent experiments done in triplicate. (B) Left panel. Apoptotic thymocytes from a CD45.1 BALB/c mouse were injected i.p. into CD45.2 BALB/c recipients and 1 h later the peritoneal cells were recovered by lavage. The fraction of CD45.1+CD45.2+BMQC+ cells was determined. Right panel. The relative apoptotic cell uptake on a per macrophage basis was compared from the mean fluorescence intensity (MFI) of the BMQC+MHC IIhi and of the BMQC+MHC IIlo populations (mean ± SD, n = 4). ***p < 0.001 by Student t test. (C) The experiment was repeated using FITC-labeled E. coli in place of apoptotic cells (mean ± SD, n = 4). **p < 0.01, ***p < 0.001 by Student t test.

Inflamed peritoneum was determined 1 d later (Fig. 5B). The results show that the conversion of the monocytes into the MHC IIlo and MHC IIhi macrophage populations is unaffected by the route of entry. To test whether this also holds true for inflammation induced by a polymicrobial peritonitis, we repeated this experiment using cecal bacteria in place of thioglycollate. As shown in Fig. 5C, the fate of the transferred monocytes 3 d later is the same after i.p. as after i.v. transfer and in both cases the large majority of the CD45.1 peritoneal macrophages are of the MHC IIhi type (Fig. 5C). We conclude that the development of the macrophages within the peritoneum is independent of whether transfer is by the i.p. or the i.v. route.

To compare monocyte development in infected and in noninfected mice we transferred BALB/c CD45.1 monocytes i.p. into BALB/c CD45.2 animals. The recipients were either untreated or were infected with CC at the time of monocyte transfer. The results show that the CD45.1.1 peritoneal macrophages recovered on day 3 from noninfected animals are of the MHC IIhi type (Fig. 6A), whereas in the infected animals they are of the MHC IIlo type (Fig. 6B). Infection thus radically alters the populations of macrophages recovered in the peritoneal wash.

**IL-10 is required for the appearance of MHC IIlo macrophages in the peritoneum postinfection**

Because neutrophils move rapidly into the peritoneum postinfection (Fig. 7A, left), and once activated produce large amounts of IL-10 (19, 20), we asked whether this cytokine might play a role in determining the nature of the macrophage populations appearing in the peritoneum. We first measured the concentration of IL-10 in the peritoneal wash of BALB/c mice over the course of infection. IL-10 reaches a peak concentration 1 d postinfection and thereafter falls back to low levels (Fig. 7A, right). A causal relationship between IL-10 and the appearance of particular macrophage populations was suggested by the fact that the formation of the MHC IIlo population postinfection with CC is very inefficient both in the IL-10–deficient animals (Fig. 7B) and in the IL-10R1–deficient mice (data not shown). Crucially, application of exogenous rmIL-10 during the initial phase of the infection in IL-10–deficient mice restored their ability to form the MHC IIlo population (Fig. 7B). Nevertheless, in uninfected mice the situation is quite different. When we compared the blood monocyte populations of uninfected BALB/c and BALB/c IL-10–deficient animals (Fig. 7C, left panels), we see no gross differences in the sizes of the Ly6Clo and Ly6Chi populations, indicating that IL-10 signaling is not required to form either of these two monocyte populations. Similarly, there are no significant differences in the macrophage populations in the peritoneum of uninfected BALB/c and BALB/c IL-10–deficient animals, indicating that IL-10 signaling is not required to form and maintain these populations in the steady-state (Fig. 7C, right panels). Similarly, C57BL/6 animals carrying a targeted deletion of the IL10R1 gene showed no gross differences in their circulating monocytes or in their peritoneal wash cell populations when compared with the C57BL/6 wild-type controls (data not shown). Thus, IL-10 signaling is not
required to form the normal blood monocyte populations nor for the differentiation of these monocytes into peritoneal MHC IIlo macrophages in uninfected mice.

The macrophage populations appearing in the peritoneum differentiate in situ from monocytes

The differences between the macrophage populations arising in the peritoneal cavities of uninfected or of infected mice could be caused either by a change in the developmental fate of the monocytes or by selective apoptosis, expansion, or emigration of particular macrophage subsets. We first looked for evidence of selective apoptosis in the emerging macrophage populations in the peritoneal wash from BALB/c mice infected with 15 mg CC 3 d previously by measuring annexin V binding. In both the MHC IIhi and the MHC IIlo populations the fraction of Annexin V+ cells was 5% (three mice per group).

To look at the possibility that differential cell division induced in the macrophage populations by IL-10 might explain the differences, we recovered peritoneal wash cells from BALB/c CD45.2 mice were transferred i.p. into BALB/c CD45.1 recipients along with 15 mg CC. Three days later the peritoneal wash cells were recovered by lavage. Aliquots were stained with appropriate Abs in the presence of TruCount beads. The grafted CD45.2 cell populations remaining in the peritoneum were determined by FACS. Values shown are mean ± SD (n = 3).

FIGURE 5. Monocyte development in the peritoneum after i.v. and i.p. transfer. (A) Blood monocytes prepared from BALB/c CD45.1 mice. (B) Monocytes (1 × 10^6) were transferred i.v. (left panel) or i.p. (right panel) into BALB/c CD45.2 recipients that were given thioglycollate i.p. at the time of transfer. Transferred cells were gated using CD45.1 and their development was assessed by their acquisition of MHC II expression (n = 2). (C) Monocytes (1 × 10^6) were transferred i.v. (left panel) or i.p. (right panel) into BALB/c CD45.2 recipients that were given CC i.p. at the time of transfer. Transferred cells were gated using CD45.1, and their development to MHC IIhi and MHC IIlo peritoneal macrophages was determined at day 3 (n = 2), was done twice, with each assay being in triplicate. We conclude that neither of the peritoneal macrophage populations divides at any significant rate.

FIGURE 6. Monocyte differentiation in the peritoneum of infected and uninfected recipients. (A) Blood monocytes (1 × 10^6) from CD45.1 mice were transferred i.p. into BALB/c CD45.2 mice. Three days after transfer the peritoneal wash cells were recovered. Macrophages were gated as shown in Fig. 4A. Left panel, The donor-derived and host populations were identified by their expression of CD45.1 or CD45.2, respectively. Center panel, The differentiation of grafted monocytes was determined using F4/80 and MHC II to distinguish the MHC IIhi and MHC IIlo populations. Right panel, Four recipients were analyzed per group. The bars show the mean values. (B) A similar experiment was carried out in which the recipients were given 15 mg CC at the time of monocyte transfer. Peritoneal wash cells were recovered 3 d later. The macrophages were gated as shown (left panel) and the differentiation of grafted monocytes was determined using Abs to F4/80 and to MHC II (center panel). Right panel, Four recipients were analyzed per group. The bars show the mean values.
Finally we considered the possibility that the rates of emigration of the developing macrophage populations are strongly influenced by IL-10. We examined the absolute numbers of MHChi and MHClo cells in the peritoneum 24 h postinfection of BALB/c or congenic IL-10–deficient mice with 2 mg CC. This time point was chosen because it is the peak of IL-10 concentration in the peritoneal wash (Fig. 7A). In the wild-type animals we recovered 1.7 \times 10^6 cells (SD, 0.45 \times 10^6; n = 4) whereas in the IL-10 animals the number of cells recovered was 3.2 \times 10^6 (SD, 1.95 \times 10^6; n = 4). These values are not significantly different (p > 0.05). We conclude that the shaping of the macrophage populations that appear postinfection is caused by an IL-10–dependent effect on monocyte differentiation within the affected peritoneum.

**IL-10 is essential to direct monocyte differentiation to the MHC II^lo macrophage fate in infected mice**

Were IL-10 to direct monocyte differentiation into MHC II^lo macrophages in infected animals, then transfer of wild-type monocytes into an infected wild-type recipient early in infection, when the IL-10 concentration is high, should permit the formation of the MHC II^lo macrophages from the donor cells. This is what happens (Fig. 8A). However, when wild-type monocytes were transferred into an infected wild-type recipient late in infection, when the IL-10 concentration has fallen (Fig. 7A), then the donor monocytes no longer form MHC II^lo macrophages (Fig. 8B). Furthermore, when wild-type monocytes are transferred into an IL-10R1–deficient recipient early in infection, then the donor but not the recipient monocytes differentiate to MHC II^lo macrophages (Fig. 8C).

A role for IL-10 in directing monocytes to the MHC II^lo fate might explain why in the normal peritoneum, which contains no detectable IL-10, monocytes differentiate into MHC II^hi macrophages (Fig. 6A). This predicts that the application of IL-10 to the normal peritoneum would enable the differentiation of monocytes to MHC II^lo macrophages. As shown in Fig. 9A, i.p. transfer of CD45.1 monocytes into a CD45.2 recipient resulted in the formation of MHC II^hi macrophages only. However, when the same procedure was carried out with animals that were supplemented with rmIL-10, then some 10% of the monocytes were induced to form MHC II^lo macrophages (Fig. 9B).

**Bystander IL-10R1–deficient monocytes can develop into MHC II^lo macrophages in the presence of IL-10 signaling-competent monocytes**

The data shown in Fig. 8 demonstrate that wild-type monocytes transferred i.p. into an IL-10R1–deficient mouse are driven by IL-10 to develop into MHC II^lo macrophages. However, when the number of wild-type monocytes transferred into an IL-10R1–deficient host was increased by an order of magnitude, then substantial numbers of host-derived MHC II^lo macrophages were now generated (Fig. 10A). Thus, increasing the number of IL-10-responsive donor monocytes injected into the peritoneum permits a bystander effect to drive the IL-10 nonresponsive host monocytes into the MHC II^lo macrophage differentiation path. A similar effect was seen when monocytes from an IL-10R1–deficient mouse were transferred into the peritoneum of a wild-type animal infected at the time of cell transfer. In this case the small number of IL-10R1–deficient monocytes was in the presence of a large number of wild-type monocytes flooding into the infected peritoneum.
peritoneum. Under these conditions the IL-10R−deficient cells were converted with high efficiency into MHC IIlo macrophages (Fig. 10B).

Discussion

Two distinct macrophage populations are present in the peritoneal wash fraction of normal uninfected mice (21). We show that both of these populations phagocytose bacteria but that they differ in other properties. The first population consists of macrophages that express large amounts of MHC II. They are able to present Ag to T cells, but show little capacity to phagocytose apoptotic cells. Adoptive transfer experiments demonstrated that this MHC IIhi population has a \( t_1/2 \) of \( ∼ 3 \) d. Blood monocytes transferred into the normal peritoneum differentiate almost exclusively into these MHC IIhi macrophages. The second peritoneal macrophage population has a distinctly lower level of expression of MHC II. These MHC IIlo cells have a longer \( t_1/2 \) in the peritoneal wash than do their MHC IIhi counterparts and they have a higher capacity to phagocytose apoptotic cells. They are, however, inefficient at presenting Ag to T cells. How these two different populations are established and maintained in normal uninfected animals remains to be determined.

Rapid changes in the peritoneal macrophage populations are seen after polymicrobial infection with cecal bacteria. Within 1 h infection all macrophages disappear from the peritoneal wash (22). The function of this “macrophage disappearance reaction” is not understood, although it is likely that these peritoneal wash cells act as a strategic reserve that is mobilized on infection. We show that these cells do not thereafter return to the peritoneal wash fraction and hence do not contribute to the MHC IIhi and MHC IIlo cell populations that are present in the peritoneal wash 3 d postinfection. Instead, the cells that have disappeared are replaced by new populations that develop from blood monocytes.

It is highly plausible that the differentiation of monocytes into the various types of tissue macrophage will be governed by the cytokine milieu in the tissue. In the case of the peritoneum of a normal uninfected mouse the cytokine milieu favors the development of the MHC IIhi macrophages. The factors involved remain to be elucidated, although it is clear that IL-10 is not required, as mice carrying a targeted deletion of the \( IL10 \) gene and those with a targeted deletion of the \( IL10R1 \) gene have normal complements of both the MHC IIhi and MHC IIlo populations. This dominant milieu in the normal peritoneum is robust, yet nevertheless injection of recombinant IL-10 does modulate the dynamics of macrophage population formation and leads to a modest excess of MHC IIlo cells developing in the peritoneal wash. In contrast, the situation in the inflamed peritoneum is quite different, for here IL-10 becomes the dominant mediator and directs the influxing monocytes to the MHC IIlo macrophage form.
FIGURE 10. IL-10R1–deficient monocytes can be activated by a bystander effect. (A) Monocytes (1.3 × 10^6) from C57BL/6 wild-type CD45.1 were transferred along with 2 mg CC into the peritoneum of C57BL/6 CD45.2 IL-10R1–deficient recipients. Peritoneal wash cells were analyzed 3 d later (n = 2). (B) Monocytes (1 × 10^6) from C57BL/6 CD45.2 IL-10R1–deficient mice were transferred along with 2 mg CC into C57BL/6 CD45.1 recipients. Peritoneal wash macrophages were analyzed 3 d later. The bars show the mean values for three recipients per group. *p < 0.05, **p < 0.01 by Student t test.

IL-10 might orchestrate the extent to which the MHC II^hi and MHC II^lo macrophage populations are represented in the peritoneal wash in a number of different ways. It might act by preferentially inducing apoptosis in the MHC II^lo population, by causing the MHC II^lo population to expand by cell division, by stimulating selective emigration of the MHC II^lo population from the peritoneum, or by influencing the direction of monocye differentiation. We found no evidence of preferential apoptosis nor did we find any evidence that either of the peritoneal macrophages undergoes rapid cell division. The number of monocyte-derived cells in an IL-10–deficient animal was not significantly different from that in a wild-type mouse, which suggests that IL-10 does not induce any major degree of selective emigration of macrophages from the peritoneum. Although one or more of these processes may well contribute to the shaping of the macrophage pool, we conclude that IL-10 exerts its major effect by affecting the direction of monocye differentiation in the inflamed peritoneum.

The early phase of a peritoneal infection with cecal bacteria is characterized by a sharp but transient rise in the concentration of IL-10 in the peritoneal cavity. Murine neutrophils, which flood into the peritoneal cavity in large numbers, are a rich source of IL-10 (19, 20) although other cell types may well also contribute to the production of this cytokine (23, 24). In this early period, when the IL-10 concentration in the peritoneum is high, the macrophages formed are of the MHC II^lo type, whereas later, when the IL-10 concentration has fallen, the macrophages formed are of the MHC II^hi type. A direct involvement of IL-10 in the formation of the MHC II^lo macrophage population in infected animals is demonstrated by the lack of formation of these cells in animals that are IL-10 signaling incompetent owing to a deficiency either of the IL10 or of the IL10R1 gene. Furthermore, when an infected IL-10–deficient mouse was supplemented with rmIL-10, MHC II^lo macrophages were now efficiently formed.

To test whether the IL-10 must interact directly with monocytes to induce their differentiation into MHC II^lo macrophages, we transferred wild-type monocytes into an IL-10R1–deficient host. In this setting the recipient’s own cells can produce IL-10 but only the donor cells can respond to it. To avoid overloading the system we transferred only 1 × 10^5 wild-type donor monocytes because this is roughly equivalent to the number of monocytes available in the murine circulation. These transferred monocytes differentiated into MHC II^lo macrophages whereas the recipient’s monocytes differentiated into MHC II^lo macrophages. However, increasing the number of IL-10 wild-type monocytes transferred by a factor of 10 permitted the recipient’s IL-10 signaling-incompetent monocytes to be driven to the MHC II^lo fate. This indicates that in the presence of a high number of wild-type monocytes the IL-10R1–deficient cells may be driven to differentiate into MHC II^lo macrophages by a bystander effect. This may be formally analogous to the situation in T cell activation, where Ag activated T cells secrete IL-2, which acts back on the secreting cell in an autocrine fashion to drive them into proliferation but that can also stimulate bystander cells in a paracrine fashion. We envisage that in an infected animal the activation of monocytes by IL-10 results in their synthesizing a mediator that is released and that then acts back on the monocytes to direct their differentiation to the MHCII^lo form. The presence of sufficient signaling-competent monocytes will thus suffice to drive signaling-incompetent bystander monocytes to differentiate into MHCII^lo macrophages. In line with this model we observed that when a small number of signaling-incompetent monocytes from IL-10R1–deficient animals were transferred into the peritoneum of an infected wild-type recipient, in which a large number of endogenous wild-type monocytes were differentiating into MHC II^lo macrophages, then the donor monocytes now also developed into MHC II^lo macrophages.

This IL-10–based switch in monocyte fate provides a means by which the course of the peritoneal infection can be tracked and optimally countered. During the early phase of the peritonitis model, a large number of neutrophils enter the peritoneum and concomitantly the number of bacteria is substantially reduced. However, these activated neutrophils are doomed to die within a few hours by apoptosis (7) and their remains must be quickly and efficiently removed. For this a substantial population of macrophages with a high capacity to phagocytose apoptotic cell bodies must be made available. During this initial period the IL-10 concentration in the peritoneum is high and directs monocyte differentiation toward MHC II^lo macrophages with the requisite capacity to phagocytose apoptotic cells. As this neutrophil-dominated phase terminates and the IL-10 concentration falls, the monocytes now differentiate into MHC II^hi macrophages, which may act as APCs and present bacterial peptides to the adaptive immune system (8).

IL-10 thus emerges as the key cytokine regulating the developmental fate of monocytes in the milieu of the infected peritoneum. It provides a means by which the immune system can optimize the host immune response to a potentially lethal peritoneal infection and respond appropriately to the rapidly changing nature of the infectious challenge.

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References


