Identification of macrophages and dendritic cells in the osteopetrotic (op/op) mouse

Margit D. Witmer-Pack1, Derralynn A. Hughes2, Gerold Schuler3, Linda Lawson4, Andrew McWilliam2, Kayo Inaba5, Ralph M. Steinman1 and Siamon Gordon2

1Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021, USA
2Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, UK
3Dermatology, University of Innsbruck, Innsbruck A6020, Austria
4Department of Pharmacology, Mansfield Road, Oxford OX1 3RE, UK
5Department of Zoology, Kyoto University, Kyoto 696, Japan

SUMMARY

We used a panel of monoclonal antibodies and immunocytochemistry to identify macrophages and dendritic cells in mice that are deficient in macrophage colony stimulating factor (M-CSF or CSF-1) because of the recessive osteopetrotic (op/op) mutation. Prior work had shown that osteopetrosis is associated with a lack of osteoclasts, phagocytic cells required for remodelling in bone. Additional macrophage populations proved to be very M-CSF dependent. op/op mice had few and sometimes no peritoneal cavity phagocytes, splenic marginal zone metallophils, and lymph node subcapsular sinus macrophages. Other populations, however, reached substantial levels in the absence of M-CSF, including phagocytes in the thymic cortex, splenic red pulp, lymph node medulla, intestinal lamina propria, liver (Kupffer cells), lung (alveolar macrophages) and brain (microglia). Dendritic cells, which are specialized accessory cells for T-dependent immune responses and tolerance, were readily identified in skin and in the T-dependent regions of spleen, lymph node and Peyer’s patch. The identification of dendritic cells utilized antibodies to MHC class II products and four different antigens that are primarily expressed by these accessory cells. Our findings indicate that only a few macrophage populations are critically dependent upon M-CSF in vivo. With respect to dendritic cells, the data are consistent with prior in vitro work where it was noted that GM-CSF but not M-CSF supported dendritic cell viability, function and growth.

Key words: macrophage, dendritic cell, osteopetrosis, M-CSF

INTRODUCTION

The osteopetrotic mutation in mice is a recessive lesion in the gene for macrophage colony stimulating factor, M-CSF or CSF-1 (Yoshida et al., 1990). The failure to produce M-CSF is critical at the level of the monocyte-derived osteoclast, the absence of which leads to impaired bone remodelling (Kodama et al., 1991). As a result, there is a deficiency in tooth eruption so that young op/op must be maintained on a soft diet to survive. Administration of M-CSF in vivo restores bone resorption in the op/op mouse (Felix et al., 1990a).

The op/op mouse provides an opportunity to look at the contribution of M-CSF to macrophages and other cell types in vivo. This is of some interest with respect to the need for M-CSF by both macrophages and dendritic cells. In vitro, M-CSF can maintain the viability and function of macrophages isolated from most tissues (Stanley et al., 1983; Tushinski et al., 1982). In contrast, the cytokine is not known to have an effect on dendritic cells in vitro. Dendritic cells are specialized antigen-presenting cells for T-cell immunity and tolerance in both tissue culture and whole animal models. While GM-CSF stimulates dendritic cell growth, viability and function (Heufler et al., 1987; Inaba et al., 1992a, 1993; MacPherson, 1989; Naito et al., 1989; Scheicher et al., 1992; Schuler and Steinman, 1985; Witmer-Pack et al., 1987), M-CSF is without apparent effect.

We have used a panel of monoclonal antibodies and immunocytochemistry to look for macrophages and dendritic cells in several tissues of op/op and op/+ mice. We find that M-CSF-deficient animals show a profound loss of a few select macrophage populations which is independent of the age of the animals studied. Macrophages are readily detectable in most sites, as is the case for dendritic cells in epidermis and lymphoid organs.

MATERIALS AND METHODS

Mice

We studied three different shipments from the op/op colony at the Jackson Labs, Bar Harbor, ME (ages 4-6 weeks), and two sets
Table 1. Monoclonal antibodies used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4/80</td>
<td>Macrophage</td>
<td>Hume et al. (1983)</td>
</tr>
<tr>
<td>SER-4</td>
<td>Sialoadhesin on stromal macrophages</td>
<td>Crocker et al. (1989)</td>
</tr>
<tr>
<td>3D6</td>
<td>Similar to SER-4</td>
<td>van den Berg et al. (1992)</td>
</tr>
<tr>
<td>FA-11</td>
<td>Macroglobulin of macrophages</td>
<td>Rabinowitz et al. (1991)</td>
</tr>
<tr>
<td>B2-1</td>
<td>MHC class II</td>
<td>Witmer and Steinman (1984)</td>
</tr>
<tr>
<td>N22</td>
<td>MHC class II</td>
<td>Metlay et al. (1990)</td>
</tr>
<tr>
<td>RA3-3A1</td>
<td>B220 antigen on B cells</td>
<td>Coffman and Weissman (1981)</td>
</tr>
<tr>
<td>RB6</td>
<td>Granulocyte antigen</td>
<td>None</td>
</tr>
<tr>
<td>53-67</td>
<td>CD8 T-cell antigen</td>
<td>Ledbetter and Hertzenberg (1979)</td>
</tr>
<tr>
<td>NLDC-145</td>
<td>Interglutinating (dendritic) cells</td>
<td>Kraal et al. (1986)</td>
</tr>
<tr>
<td>N418</td>
<td>CD11c integrin: abundant on dendritic cells</td>
<td>Metlay et al. (1990)</td>
</tr>
<tr>
<td>2A1</td>
<td>Granule antigen of dendritic and B cells</td>
<td>Unpublished details</td>
</tr>
<tr>
<td>M342</td>
<td>Granule antigen of dendritic and some B cells</td>
<td>Agger et al. (1992)</td>
</tr>
</tbody>
</table>

from Dr R. Stanley’s colony (Albert Einstein Medical College, New York, NY), (ages 8 weeks and 8 months).

Peritoneal cells

These were rinsed from the peritoneal cavity with PBS. Cytospins were prepared on a Shandon cytocentrifuge (Sewicki, PA), stored under desiccant and fixed in acetone prior to immunolabeling (see below).

Epidermal sheets

Ear halves were treated with ammonium thiocyanate prior to peeling off the epidermal sheets for acetone fixation, staining and counting as previously described (Romani et al., 1985).

Sections of lymphoid and nonlymphoid organs

Fresh organs were sectioned directly after embedding in OCT cryoprotection medium, or the organs were obtained from mice that were fixed by perfusion (Hume et al., 1983) with periodate-lysine-paraformaldehyde (McLean and Nakane, 1974).

Immunolabeling

A panel of monoclonal antibodies was used (Table 1). After applying hybridoma supernatant for 45-60 min, rat mAbs were visualized with either biotin-rabbit, anti-rat Ig and an avidin-peroxidase complex (ABC, Elite Labs, Peterborough, UK) or peroxidase-mouse, anti-rat IgG (Boehringer Mannheim, Indianapolis, IN). Hamster mAbs were identified with peroxidase-rabbit F(ab')2 antibody Ig (Accurate Chemical and Scientific, Westbury, NY). Two-color labeling has been described (Agger et al., 1992).

RESULTS

Macrophage populations that are defective in op/op mice

Certain populations of macrophages were profoundly reduced in op/op mice. Peritoneal macrophages were rare, as identified by cytology or by staining with the anti-macrophage monoclonal antibody F4/80 (Hume et al., 1983), (Fig. 1, top). Lymphoid organ phagocytes that stain strongly with the SER-4 and 3D6 monoclonal antibodies to sialoadhesin (Crocker and Gordon, 1989; van den Berg et al., 1992) were undetectable. In spleen, these monoclonal antibodies stain the marginal metallophilis at the periphery of the white pulp nodule (Fig. 1c), while in node, these monoclonal antibodies stain the macrophages that lie beneath the subcapsular sinus (Fig. 1e). SER-4 and 3D6 staining were totally absent in the spleen and lymph node of op/op mice (Fig. 1d,f). This likely reflected a loss of cells rather than of the 3D6/SER-4 antigen, since the CD11b molecule that is found on the same populations (Witmer and Steinman, 1984), using mAbs M1/70 (Beller et al., 1992) and 5C6 (Rosen and Gordon, 1987), was also absent in the same regions of spleen and node (not shown). 3D6 and CD11b antigens were detectable on gut macrophages of op/op mice (see below).

Macrophage populations present in substantial numbers in op/op mice

Several populations of macrophages were present in op/op mice in substantial numbers relative to control op/+ littermates. In splenic red pulp, abundant phagocytes were detected using either F4/80 (Fig. 2a and b) or FA-11 (not shown) monoclonal antibodies. It appeared that the amount of red pulp was increased in op/op mice. This is consistent with the need for extramedullary hematopoiesis as a result of a severe reduction in bone marrow macrophages in op/op mice (Felix et al., 1990b). In liver, Kupffer cells lining the liver sinusoids were typically decreased relative to op/+ controls (Fig. 2c,d), although the number of F4/80- and FA-11-positive cells varied considerably between different fields of op/op sections (not shown). In both lung and kidney, macrophages were readily detected in op/op mice, although their numbers appeared reduced relative to controls. In gut (Fig. 2e,f), where macrophages are particularly abundant in lamina propria (Lee et al., 1985), both F4/80 and 3D6 monoclonals stained numerous phagocytes in the op/op mice. In thymic cortex and in brain, the incidence of phagocytes staining with F4/80 (Fig. 2g,h) and FA-11 (not shown) in op/op mice was comparable to controls.

Table 2. Frequency of Langerhans cells in epidermal sheets

<table>
<thead>
<tr>
<th>Mouse #</th>
<th>LCs/mm²</th>
<th>Control/op.op</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>op.op 1</td>
<td>532</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>422</td>
<td>1.5</td>
</tr>
<tr>
<td>Control 1</td>
<td>785</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>730</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>op.op 1</td>
<td>565</td>
<td>1.7</td>
</tr>
<tr>
<td>Control 1</td>
<td>987</td>
<td></td>
</tr>
</tbody>
</table>

The above data are the mean number of Langerhans cells (LCs)/mm² obtained from counts of triplicate specimens, each having 5-7 fields of 0.272 mm² (counts of about 100-1200/field). Standard errors were <15% of the mean and are not shown. In experiment 1, the animals were 3 months of age, and in experiment 2, 5 weeks of age.
**Epidermis**

The epidermis of skin contains a population of stellate Langerhans cells that express some F4/80 antigen (Hume et al., 1983). When maintained in culture, these cells take on many distinctive traits of dendritic cells of lymphoid organs, including very low levels of F4/80 (Schuler and Steinman, 1985). In epidermal sheet preparations, the Langerhans cells of op/op mice were normal in appearance (Fig. 3), but their numbers were only about 60% of controls (Table 2).

**The interdigitating cells (dendritic cells) of the T-cell areas**

T and B cells circulate through distinct regions of peripheral lymphoid tissues (spleen, lymph node and Peyer’s patch). The T-cell area contains a network of distinctive interdigitating cells (Fossum, 1989; Veldman and Kaiserling, 1980) which are similar in many respects to isolated dendritic cells. The similarities include surface antigens (MHC class II, CD11c integrin, NLDC-145 antigen) as well as the intracellular granule antigens detected by M342 and...
2A1 (Agger et al., 1992; Kraal et al., 1986; Metlay et al., 1990; Witmer and Steinman, 1984). Interdigitating cells react weakly or not at all with several phagocyte monoclonal antibodies, such as F4/80 and SER-4 anti-macrophage and RB-6 anti-granulocyte (Witmer and Steinman, 1984). To identify dendritic cells in the T-cell areas, we applied this panel of reagents to sections of spleen, lymph node and Peyer’s patch from op/op and op/+ mice.

Fig. 2. Macrophages (arrows) in several organs of op/op mice, as identified with the F4/80 anti-macrophage reagent (all panels except f, which is stained with 3D6 anti-sialoadhesin). ×100. (a) op/+ spleen; (b) op/op spleen; (c) Kupffer cells in op/+ liver; (d) Kupffer cells (e.g. arrows) in op/op liver; (e,f) op/op intestinal lamina propria; (g) macrophages in op/op thymic cortex (e.g. arrows) (h) microglia in op/op brain (e.g. arrows).
For each organ, the appropriate mAbs (Table 1) identified dendritic cells in what appeared to be normal numbers in op/op mice.

In spleen, sections were double labeled to simultaneously visualize dendritic cells (hamster mAb M342, an intracellular granular antigen in dendritic cells; Agger et al. (1992) and phagocytes (F4/80 and SER-4 anti-macrophage; RB6 anti-granulocyte). M342 stained profiles in the periarterial sheaths comparably in op/op versus op/+ mice (Fig. 4). SER-4 bearing macrophages were absent in op/op (Fig. 4a,b), whereas red pulp macrophages (F4/80, Fig. 4c,d) and granulocytes (RB6, Fig. 4e,f) were evident.

Single-color immunoperoxidase labels for dendritic cells are shown for lymph node and Peyer’s patch (Fig. 5). Dendritic cells were evident in the T-cell areas of op/op mice regardless of the monoclonal antibody that was used for their identification. Dendritic cells were also evident in the thymic medulla (not shown) comparable to control thymic medulla as described elsewhere (Agger et al., 1992).

**DISCUSSION**

Osteopetrosis is an inherited metabolic bone disease, characterized by skeletal sclerosis due to a failure of bone resorption and modelling as a result of a functional defect.
of osteoclasts (Brown and Dent, 1971). op/op mice are
toothless, smaller in size, have extensive skeletal defects
and have low numbers of peripheral macrophages (Marks
and Lane, 1976; Wiktor-Jedrzejczak et al., 1982). The defi-
ciency of mononuclear phagocytes results from a complete
absence of circulating and tissue M-CSF (Wiktor-Jedrzejeczak et al., 1990) resulting from an inactivating mutation
in the M-CSF gene (Yoshida et al., 1990). M-CSF is syn-
thesized by diverse cell types including fibroblasts, bone-
marrow stromal cells, osteoblasts and monocytes (reviewed
by Roth and Stanley, 1992), and its receptor is primarily
expressed on mononuclear phagocytes (Guilbert and Stan-
ley, 1980), which survive, proliferate and perform various
differentiated functions under the control of this growth
factor. In vitro, M-CSF can synergise with a number of
cytokines including IL-3 (Chen and Clark, 1986), GM-CSF

Fig. 4. Double labels of spleen from op/op versus op/+ mice, stained with the M342 monoclonal to dendritic cell granules (alkaline
phosphate anti-hamster Ig, blue), and monoclonals to phagocytes (peroxidase anti-rat Ig, brown). (a,c and e) are from op/+ mice while
(b,d and f) are from op/op mice. Each micrograph is from a comparable region of spleen in which the periarterial sheaths of T area is on
the left (T), the B-cell follicle in the middle (B), and the macrophage-rich marginal zone (M) on the right. ×245. (a,b) SER-4 anti-
marginal zone metallophil. In op/op, endogenous peroxidase in eosinophils (arrows) marks the outside of the marginal zone, which fails
to stain with SER-4 or 3D6 to sialoadhesin. (c,d) F4/80 anti-red pulp macrophage. (e,f) RB-6 anti-granulocyte.
(Caroccilo et al., 1987) and TNFα (Branch et al., 1989), so that the sensitivity of certain macrophage (MØ) populations to M-CSF may also depend on the actions of other cytokines.

Our findings with a large panel of monoclonal antibodies applied to several tissues of M-CSF-deficient op/op mice, indicate that certain macrophages are very M-CSF dependent whereas others reach substantial levels in the absence of this growth factor (summarised in Table 3). The dependent cells are macrophages within the peritoneal cavity, the marginal zone metallophils of spleen, and phagocytes beneath the subcapsular sinus of lymph node (Fig. 1). A cytokine-like GM-CSF also stimulates the production and function of macrophages and might be expected to substitute for M-CSF. If so, the M-CSF-dependent macrophage populations in peritoneal cavity and certain lymphoid regions may not be sensitive to GM-CSF in vivo. Other phagocyte populations in splenic red pulp, lymph node medulla, liver, lung, intestine and brain likely are utilizing GM-CSF or perhaps other cytokines to achieve the sizable

Fig. 5. Single color label for dendritic cells in the lymph node cortex (a-c) and Peyer’s patch (d-f) of op/op mice. ×160. (a) B21-2 anti-MHC class II stains B cells in follicles (B) and dendritic cells in the T area (T). (b) N418 anti-CD11c primarily stains dendritic cells in the deep cortex (T) as well as a few profiles above the follicles (B). (c) M342 anti-dendritic cell granule stains the T but not B areas. (d) B21-2 anti-MHC class II stains the follicles (B) and the dendritic cells of the interfollicular T areas (T). (e) N418 anti-CD11c stains dendritic cells in the interfollicular regions (T) and cells around the follicles (B). (f) M342 anti-dendritic cell granule only stains the T areas (arrowheads).
levels that are seen in op/op mice (Figs 2, 4). Immunocytochemistry with several MØ-restricted monoclonal antibodies indicated the total or partial depletion of specific MØ subpopulations as opposed to the down-regulation of individual markers. This is especially evident in the spleen and lymph node, where specialized marginal zone and subcapsular MØ are lost but red pulp and medullary MØ retained. In both instances, the MØ subpopulation lost is phenotypically distinct from that remaining and can be identified by its unique combination of surface markers, for example marginal metallophil MØ are SER+, CD11b+, F4/80- compared to red pulp MØ which are F4/80+, FA.11+, CD11b-, SER-;im. The heterogeneity is likely to reflect tissue localization, differentiation and maturation in response to cell-derived and environmental signals.

Dendritic cells also reached substantial and possibly normal numbers in op/op mice, as assessed by antibodies to MHC class II products and to four other antigens that are primarily expressed by dendritic cells (Figs 3-6). These findings are consistent with prior in vitro work where GM-CSF, but not M-CSF, supported dendritic cell viability and function (Heufler et al., 1987; Witmer-Pack et al., 1987).

No age-related differences in the frequency of dendritic cells and macrophages were observed in mice aged 5-6 weeks, 3 months and 8 months, whereas Langerhans cells showed a slight age-dependent increase: the ratio of control littermate epidermal Langerhans cells to those of the op/op is 1.7 at 5 weeks compared to 1.5 at 3 months.

The lineage of dendritic cells has not been worked out fully. Recent studies illustrate extensive production of these cells in bone marrow and blood cultures in response to GM-CSF but not M-CSF (Inaba et al., 1992a,b). Studies in semisolid colony-forming systems indicate that the dendritic cell arises from an MHC class II negative, common precursor to macrophages and granulocytes (Inaba, 1993). This pathway seems to be M-CSF independent in vivo.

REFERENCES


op/op mouse macrophages and dendritic cells  


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