Clin. exp. Immunol. (1987) **70**, 515–519

Inflammatory cells in murine visceral leishmaniasis express a dendritic cell marker

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(*Accepted for publication 10 July 1987*)

SUMMARY

Immunohistological studies of the murine liver following *Leishmania donovani* infection have been performed. We describe here the identification of cells expressing a recently defined dendritic cell marker, as detected by monoclonal antibody NLDC 145. Such cells are numerous in the developing inflammatory foci but are not detected individually at any other site in the infected liver. This finding suggests that mature tissue DC are not recruited during infection and that expression of this antigen is under precise microenvironmental control.

Keywords dendritic cells cell surface antigens inflammation *Leishmania donovani*

INTRODUCTION

The requirement for a non-lymphoid accessory cell (AC) in the activation of T lymphocytes is now well documented and a variety of cells have been shown to perform in this respect. Attention has principally focussed on members of the dendritic cell series (DC) (as defined by Tew, Thorbecke & Steinman (1982), and with the exception of follicular dendritic cells), and on the macrophage, in its many states of differentiation and activation (reviewed in Steinman & Nussenzweig, 1980; Unanue, 1984). Two questions have dominated this field of study; the relative functional capacity of these different cell populations and their inter-relationship.

Under many experimental conditions, DC are very potent stimulators of T cell responses compared to resting macrophages even when variables such as relative class II expression are taken into account (Sunshine, Katz & Feldmann, 1980; Kaye, Chain & Feldmann, 1985). There is also compelling evidence for macrophage accessory cell activity especially when recruited to the site of antigenic challenge during an immune response (Beller, 1984; Kaye & Feldmann, 1986). DC and macrophages may thus be considered to represent primary and secondary AC populations, in terms of the temporal regulation of the response.

The relationship between DC and macrophages has been less easy to resolve. The application of monoclonal antibodies (MoAb) to human tissue or isolated cell populations generally supports the concept of inter-differentiation between AC types (Goodyal & Isaacson, 1985; Poulter *et al.*, 1984). In the murine system, a pan macrophage marker, F4/80 has long been available (Austyn & Gordon, 1981) but only recently has a marker for tissue DC been described which is suitable for immunohistochemical use (Kraal *et al.*, 1986). This rat MoAb, NLDC 145, recognizes a 145 kD

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molecule almost exclusively on Langerhans' cells, veiled cells and interdigitating cells in the naive animal.

As part of a study of the immunohistological changes within the AC and lymphocyte compartments during murine visceral leishmaniasis (to be described in detail elsewhere) this MoAb was used to look for a possible redistribution of DC to the infected liver.

MATERIALS AND METHODS

Animals and infections. C57BL/10 ScSn mice aged 6–8 weeks old (Harland Olac Ltd, Bicester UK) were infected intravenously with 10^7 amastigotes of *Leishmania donovani* LV9 as described elsewhere (Kaye, 1987). At various times after infection, groups of 2–3 mice were killed by cervical dislocation, and their spleens and livers removed.

Immunohistochemistry. Tissues were snap frozen in iso-pentane in liquid nitrogen and sectioned immediately or after storage at −70°C.

Cryostat sections of 5–8 μm were air-dried, fixed in acetone for 10 min at 4°C and stained with an avidin-biotin–peroxidase technique, with steps to block endogenous avidin binding activity (Wood & Warnke, 1981). Sections were sequentially incubated at room temperature with avidin (200 μg/ml in TBS; 15 min), biotin (100 μg/ml in TBS; 15 min), normal lamb serum (50% v/v in TBS; 20 min), primary antibody (30–60 min); biotin labelled sheep anti rat IgG (Amersham UK, 1/100 in TBS + 1% NMS; 45 min), 0·3% H_2O_2 in methanol (30 min to block endogenous peroxidase activity) and avidin peroxidase (Sigma 1/100 in TBS + 1% normal lamb serum; 30 min). Enzyme was visualised using 3′, 3′-diaminobenzidine tetrahydrochloride (DAB; 0·5 mg/ml in TBS + 0·01% H_2O_2) for 5–10 min followed by enhancement with 1% Cu_2SO_4 in saline. Sections were counterstained with either haematoxylin or methylene blue (1% in water), processed through alcohols and xylene and mounted in DPX.

Primary antibodies were culture supernatants produced from hybridoma cell lines supplied by the American Type Culture Collection (Maryland, USA) or as generous gifts from Dr Siamon Gordon (Sir William Dunn School of Pathology, Oxford) and Dr Georg Kraal (Free University, Amsterdam). Their specificity and staining pattern was confirmed by staining tissues from normal uninfected mice, and are given in Table 1. Control staining, performed using normal rat serum (1/20 in TBS) or by omitting primary antibody, was uniformly negative.

RESULTS

Unlike the secondary lymphoid organs, where a large and heterogeneous group of AC occur, the normal liver presents a much more restricted picture. Mature dendritic cells, as defined by NLDC

Table 1. Specificity of MoAb used and reactivity in normal liver

Antibody	Specificity	Liver reactivity	Reference
NLDC 145	LC, VC, IDC, TE	Negative	Kraal *et al.* (1986)
F4/80	MPS	Kupffer cells	Austyn & Gordon (1981)
F7/4	PMN, MO, AMØ	Rare PMN	Hirsch & Gordon (1983)
M1/70	CR3	Negative	Springer *et al.* (1979)
M5/114	$I\text{-}A^{b,d,q}\text{-}E^{d,k}$	Kupffer cells	Bhattacharya, Dorf & Springer (1981)

LC, Langerhan's cell; VC, veiled cell; IDC, interdigitating cell; MPS, mononuclear phagocytes; PMN, neutrophils; MO, monocytes; AMØ, activated Ø, activated macrophages; CR3, receptor for iC3b; TE, thymic epithelium.

Table 2. Distribution of cells reactive with various MoAb 7–28 days after infection with 10^7 *Leishmania donovani* amastigotes

Antibody	PVI	SLC	Parenchyma*	Inflammatory foci†
NLDC 145	−	−	−	+
F7/4	±	−	+	+
M1/70	+	±	+	+
F4/80	±	+	+	+
M5/114	+	+	+	+

−, not detected; ±, occasionally seen; +, present in large numbers.

PVI, Perivascular infiltrate; SLC, sinusoid lining cells—predominant reactivity to KC.

*Randomly scattered in sinusoids, not associated with inflammatory accumulations.

†Accumulations of 10 or more mononuclear cells.

145 staining, are absent and classical macrophages are represented almost solely by sinusoid lining Kupffer cells, with only a rare monocyte detected in portal vessels (Table 1).

The major finding reported here is the demonstration of numerous NLDC 145$^+$ cells within the *Leishmania* infected liver. Such cells were not present in the early infiltrating population, but can be seen in increasing numbers from approximately 7 days after infection. From 7 to 28 days, the overall pattern of staining with a panel of monoclonal antibodies did not vary extensively, even though the lesions increased in size, number and complexity. This pattern is summarized in Table 2. NLDC 145$^+$ cells were present only within the developing inflammatory foci (Fig. 1), often comprising much of their sectional view. This pattern of reactivity is quite distinct from that seen with any of the other MoAb used. In particular M1/70 and F7/4 readily identify single infiltrating cells lying within sinusoids throughout the parenchyma and not apparently associated with any inflammatory foci. Similar individual cells stain with anti-class II, seen against a network of positive Kupffer cells. Within large foci, most cells were stained with all the antibodies used, implying some degree of coexpression of these markers on NLDC 145$^+$ cells.

Morphologically, cells expressing NDLC 145 antigen were large, with abundant cytoplasm but lacking the dendritic appearance seen in other tissues. Occasionally, they appeared to contain amastigotes.

DISCUSSION

The detection of NLDC 145$^+$ cells in the infected liver and their limited distribution raises a number of issues. Firstly, the inability to detect such cells in any site outside the developing inflammatory foci and yet the ease of detection of even small numbers of infiltrating M1/70$^+$ and F 7/4$^+$ cells (this report; Davies, Singleton and Blackwell, submitted) suggests that mature dendritic cells expressing NLDC 145 are not recruited to the liver during infection. As no NLDC 145$^+$ cells were detected in either blood or bone marrow (Kraal *et al.*, 1986), immature precursors do not appear to express this marker. Hence the precursor of this liver population could either be distinct from, or reside within, the otherwise defined infiltrates. M1/70 and F4/80 have already been shown to be variably expressed on different DC (Schuler & Steinman, 1986; Hume *et al.*, 1983).

Irrespective of the identity of the precursor, it is clear that the expression of NLDC 145 is exquisitely regulated by the microenvironment of the developing inflammatory focus. Similar control has been described for other markers of the dendritic series in man (Wood *et al.*, 1985; Alegre, MacDonald & Poulter, 1986) but is quite distinct from the regulation of the macrophage activation marker F7/4 (Gordon *et al.*, 1985) and the expression of MHC class II antigens. Both of

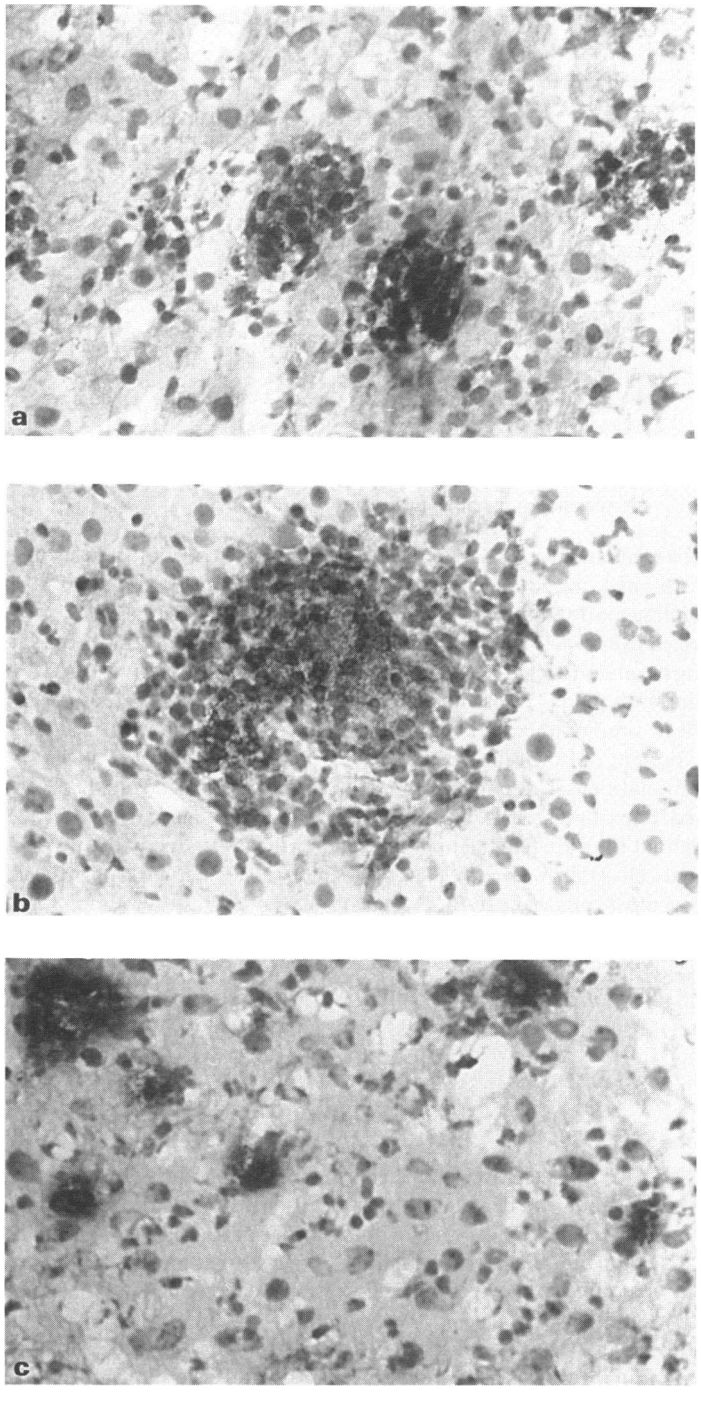

Fig. 1. Immunohistochemical demonstration of NLDC 145$^+$ cells in the *Leishmania* infected liver. (a) NLDC 145$^+$ cells in hepatic inflammatory foci of C57BL/10 mice 10 days after infection with *L. donovani* amastigotes. Note stained cells are confined to these areas. (b) NLDC 145$^+$ staining of 28 day lesion. Abundant cytoplasmic staining is evident especially on cells at the core of the focus. (c) Antigen F7/4 bearing cells resembling activated macrophages are identified not only in inflammatory foci but also individually within the parenchyma ; 10 days after infection as in (a).

these are present on cells at all sites in the infected tissue, presumably in response to more diffuse stimuli.

In view of the precise microenvironmental control governing expression of this antigen, both on the primary AC populations in the skin and secondary lymphoid organs and on cells recruited to the site of inflammation, this recent MoAb may hold greater promise in the functional assessment of AC activity than in the question of lineage. Such a suggestion is further strengthened by the known cross-reactivity of NLDC 145 with thymic epithelium (Kraal *et al.*, 1986).

I thank the many participants of the 1986 BSI Workshop on Antigen Presenting Cells for fruitful discussion, A. Singleton for histological assistance and Alison Hinchley for typing the manuscript. This work was supported by the Medical Research Council and the Wellcome Trust.

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