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Human follicular dendritic cells (FDC): a study with monoclonal antibodies (MoAb)

G. D. JOHNSON, DEBORAH L. HARDIE, N. R. LING & I. C. M. MACLENNAN
Department of Immunology, The Medical School, University of Birmingham, UK

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SUMMARY

A collection of new and established FDC-reactive MoAb has been used in an immunohistological study designed to throw light on (a) the nature of FDC (which are of unknown lineage) as judged by their sharing of antigens with other cell types and (b) the reason for the strong expression of some B cell antigens on FDC. The MoAb were tested on: (1) sections of tonsil, (2) sections of lymph nodes from four cases of non-Hodgkin's lymphoma, (3) peripheral blood cells and (4) cells of cultured haemopoietic cell lines. Only one of the ten new MoAb bound to FDC and no other component of the tissues screened. It resembled R4/23, a MoAb known to be specific for FDC. The other nine antibodies showed a range of cross-reactivity patterns involving one or more of the following: monocytes, macrophages, platelets, epithelium, endothelium and connective tissue fibres. Some of the MoAb reacted with B lymphocytes and cells of B lymphoblastoid lines but none showed the restricted FDC-staining pattern associated with MoAb which detect the CD23, P45 antigen. The findings are discussed in terms of the intrinsic or extrinsic nature of the antigens detected.

Keywords follicular dendritic cells monoclonal antibodies

INTRODUCTION

Follicular dendritic cells (FDC), first described by Maximow in 1927—'embryonal non-phagocytic reticulum cells'—are found exclusively in B cell-rich follicles of peripheral lymphoid tissue. In the germinal centres of secondary follicles their cytoplasmic processes form a dense network closely associated with the lymphoid cells. Unlike macrophages, FDC are not phagocytic but bind and retain antigen-antibody complement complexes (Nossal & Ada, 1971; Heinen, *et al.*, 1985). The immobilized complexes seem likely to provide the antigenic stimulus for B cell proliferation which is known to occur in germinal centres (Nieuwenhuis & Keuning, 1974). The origin of FDC and their relation to other cell types is not known, but there is evidence from mouse reconstitution experiments that they are not derived from precursors in bone marrow (Humphrey, Grennan & Sundaram, 1984).

FDC are readily demonstrated in tissue sections by staining with antibodies to constitutive antigens or to determinants on the passively absorbed complement or immunoglobulin. A monoclonal antibody (MoAb) has been described (R4/23) which binds to an epitope found exclusively on FDC (Naiem *et al.*, 1983). The staining with another MoAb, Tü-1, (Ziegler *et al.*, 1981) is restricted to the light (centrocytic) portion of the germinal centre, corresponding to areas

Correspondence: Dr G. D. Johnson, Department of Immunology, The Medical School, University of Birmingham, Vincent Drive, Birmingham B15 2TJ, UK.

showing 5-nucleotidase activity. This has led to the suggestion that FDC are heterogeneous and may vary in state of differentiation and function (Stein, Gerdes & Mason, 1980). A different restricted pattern of staining of FDC is seen with anti-B cell MoAb of the P45, CD23 cluster (Johnson *et al.*, 1985).

In order to obtain further information on the antigenic constitution of FDC we have studied the patterns of cross-reactivity shown by a collection of MoAb identified as FDC-reactive. These were detected in the course of screening supernates from hybridomas derived from fusions of plasmacytomas with spleen cells of mice immunized with human lymphoid cells from various sources.

MATERIALS AND METHODS

Monoclonal antibodies (MoAb)

(a) *New MoAb.* Hybridomas were generated by fusion of BALB/c plasmacytoma cells (P3-NS1/1.Ag5·1 or X63/Ag8·653) with spleen cells of BALB/c mice immunised with various cells as shown in Table 1. The procedure was essentially that of Galfré *et al.* (1977). Culture supernates were screened by immunofluorescence (IF) on preparations of the immunizing cells and on cryostat sections of tonsil. Selected hybridomas were grown up in culture and in pristane-primed mice. The ascitic fluid harvested was used in all subsequent studies. Care was taken to exclude antibodies binding to immunoglobulin determinants. These were identified by observing the blocking effect of human serum on binding of antibody to sections and by passive haemagglutination tests using red cells coated with a range of immunoglobulin antigens.

(b) *Reference MoAb.* The MoAb R4/23, which is specific for FDC (Naiem *et al.*, 1983) and the MoAb BL-13 (p140, CD21) which binds to the C3d receptor of B cells as well as to FDC (Brochier *et al.*, 1984) were used as reference antibodies throughout the work. Two MoAb representative of B cell cluster antibodies known to be reactive with FDC and identified in the Second International Workshop on Human Leucocyte Differentiation Antigens (HLDA) were also included. These were B4, a CD19, pan B antibody which reacts with a p45 antigen and MHM6, a CD23 antibody of restricted B cell specificity which reacts with a p45 antigen. These MoAb originated from the laboratories of Dr Lee Nadler and Dr Andrew McMichael respectively. Another pan-B MoAb, WR-17, was kindly provided by Dr K. Moore, Southampton.

Table 1. Immunogens and fusion partners

Hybridoma clone	Immunoglobulin isotype	Fusion Partner	Immunogen
2BD11	M	NS1/1.Ag 4·1	Hairy cell
2BF11	M		leukaemia cells
8AF7	G1	X63/Ag 8·653	EB4 Burkitt
8EB1	G1		lymphoma cell line
11CD8	G1	X63/Ag 8·653	WM-1 B-LCL
HJ7	G1	NS1/1.Ag 4·1	Synovial fluid
HJ11	G1		dendritic cells*
N2C1	G2a		
N3A4	M	X63/Ag 8·653	Tonsil cells†
N3C3	M		

* A 'light' mononuclear cell fraction provided by Dr Stella Knight.

† A cell dispersion prepared with collagenase and dispase as described by Humphrey *et al.* (1984).

(c) *Determination of isotypes of MoAb.* Heavy chain isotypes of the MoAb were determined by double diffusion in agarose using heavy chain-specific sheep anti-mouse immunoglobulin antisera and hybridoma culture supernates. If a clear-cut result was not obtained in the precipitin test MoAb were tested by immunofluorescence on tissue sections with immunoglobulin isotype-specific fluorescein conjugates as second stage reactants. The antisera and conjugates were obtained from BDS Biologicals Ltd, University of Birmingham.

Immunofluorescence (IF)

(a) *Sections.* Standard procedures, as previously described, were employed throughout (Johnson & Holborow, 1986). Cryostat sections of snap-frozen human tissues including tonsil, normal spleen skin and kidney, and lymph nodes from four patients with malignant lymphoma were cut at 6μm and fixed in acetone for 5 min at room temperature. Culture supernates were tested undiluted and ascitic fluids diluted 1 : 50 in phosphate buffered saline (PBS) containing 10% fetal calf serum. Double fluorescent staining was performed by applying paired mixtures of test antibodies and reference antibodies with different immunoglobulin heavy chain isotypes, followed by staining with paired fluorescein and rhodamine conjugates of appropriate isotype specificities. Slides were mounted in 90% glycerol, pH 8·6 containing diazabicyclooctane (DABCO) to retard fading during microscopy (Johnson *et al.*, 1982).

(b) *Cell preparations.* Mononuclear cells were separated from normal peripheral blood on Ficoll-Triosil; 1×10^6 cells were suspended in 100 μl of the antibody preparations diluted 1:25 and rotated for 30 min on ice. The cells were stained in diluted conjugate for 30 min, and finally suspended in 50% glycerol containing DABCO. A similar technique was used for detection of antigens on the surface of viable cell line cells. In addition tests were performed on acetone-fixed cytocentrifuge preparations (obtained in a Shandon Cytospin 2).

Tests were read on Leitz and Zeiss fluorescence microscopes equipped for epi-illumination.

Cell lines

The following cell lines were used: (1) SMS-SB, a pre-B cell line (sIg$^-$, cyto μ^+, CALLA$^-$, MHC class II$^+$; Smith, Dev & Shannon, 1981); (2) three Burkitt lymphoma cell lines (EB4, Raji & Daudi); (3) a B cell lymphoma line ARH-77 (sIg$^+$, MHC class II$^+$); (4) a plasmacytoid line, RPMI-8226 (low sIg, low MHC class II, free lambda chains secreted); (5) WM-1, a EBNA$^+$ line grown from the EBV-infected blood cells of a patient with Waldenström's macroglobulinaemia; (6) Ed-1 from EBV-transformed blood lymphocytes of a normal individual; (7) two T cell lines (HSB-2 and MOLT-4); (8) a Myeloid cell line, HL-60. We are grateful to Dr P. Edwards, Sutton, Dr B. Steel, Edinburgh, Professor A. Rickinson, Birmingham and Dr G. Brown, Birmingham for providing these lines. All cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum.

RESULTS

Ten MoAb derived from the fusions shown in Table 1 were selected on the basis of unequivocal staining of FDC in tonsil sections shown not to be attributable to anti-immunoglobulin specificity. The heavy chain isotypes of the MoAb are also shown in Table 1. The cellular reactivity patterns are shown in Tables 2 and 3. All ten MoAb stained FDC in sections of tonsil from three donors (Table 2). The staining was cytoplasmic and generally appeared as a granular deposit superimposed on a diffuse background; one antibody, 8AF7, gave a distinctive granular pattern without the diffuse background staining shown by the other MoAb. Mantle zone lymphocytes immediately adjacent to the FDC network usually appeared to be weakly stained, presumably due to extension of the dendritic processes beyond the germinal centre. It was established by double IF that the staining of FDC by all the new MoAb completely coincided with that obtained with the established FDC reactive antibody R4/23. All the MoAb of the CD19 and CD21 clusters from the Second International HLDA Workshop also gave a completely coincident staining pattern with R4/23 when similarly tested on tonsil sections, as already reported (Johnson *et al.*, 1985). A more restricted pattern of staining was obtained with the p45 CD23 antibodies (Table 4). This restricted pattern was not observed with any of the new MoAb.

Table 2. Reactivity of MoAb with cells in tissue sections and peripheral blood, detected by immunofluorescence

Clone name	Tonsil			Nhl lymph node				Tonsil and spleen			Peripheral blood Viable MN cells			Other reactivity
	FDC			FDC				Macrophages	Lymphocytes		Monocytes	Lymphocytes		
	1	2	3	1	2	3	4		T	B		T	B	
2BF11	++	++	++	+	++	+	+	−	−	−	−	−	−	None
N3A4	++	++	++	+	+	W	+	−	−	−	−	−	−	Strong staining of connective tissue fibres associated with adventitia of blood vessels, splenic trabeculae, skin basement membrane, vitreous membrane of hair follicles
HJ11	++	++	++	+	+	W	+	+	−	−	+	−	−	None
2BD11	+	+	+	+	+	W	W	−	VW‡	−	−	−	−	None
N3C3	++	++	+	+	+	−	+	−	−	−	−	−	−	Strong staining of minor population of DC in crypt epithelium and sub-epithelial areas. Similar cells in fetal thymus. No staining of T-zone IDC in tonsil or skin DC
11CD8	++	++	++	+	+	W	−	−	−	VW‡		VW	VW¶	None
HJ7	++	++	++	+	+	−	++	+	+§	+	+	VW	VW¶	Bright staining of fibres in dermis, ? elastin. No staining of elastic lamina of arteries
N2C1	++	++	++	+	+	W	+	W	−	−	−	−	−	Tonsil epithelium especially suprabasal layers, endothelium in blood vessels including arterioles; blood platelets
8EB1	+	++	++	D†	+	W	D†	−	−	+	−	−	+	None
8AF7	++	+	+	+	+	−	+	−	−	W	−	−	−	None
R4/23	++	++	++	+	++	+	++	−	−	−	−	−	−	None
BL13	++	++	++	+	+	+	+	−	−	+	−	−	+	None

W = weak reaction; DC = dendritic cells; IDC = interdigitating cells; MN = mononuclear cells.
* Non-Hodgkin's lymphoma (NHL). These were all low grade lymphomas of the centroblastic-centrocytic type.
† Doubtful result due to the presence of associated B cell reactivity.
‡ Very weak staining of some peripheral lymphocytes of germinal centres.
§ Predominantly staining lymphocytes in B areas but some lymphoid cells in T areas also positive.
¶ Very weak staining of some peripheral blood lymphocytes—too weak for definitive analysis.

Table 3. Binding of MoAb to viable and acetone-fixed cells of lymphoblastoid and myeloid cell lines

	Pre–B		Burkitt Lymphoma						Other B–LCL						Plasmacytoid		T cell		Myeloid	
Clone	SMS–SB		Daudi		E B 4		Raji		Ed-1		ARH-77		WM-1		RPMI-8226		HSB-2		HL-60	
name	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm
11CD8	+	+	±	−	−	−	−	−	+	+	+	+	±	±	−	−	−	−	−	−
HJ7	−	±	++	++	±	−	++	++	++	++	+++	++	+++	++	+	++	−	−	−	±
N2C1	+	++	±	±	−	±	−	−	++	±	±	−	−	++ (7%)	+++	++	−	−	−	−
8EB1	++	++	++	+	±	±	+	+	++	++	++	++	±	+ (rare)	−	++ (rare)	−	−	−	−
8AF7	++	−	++	−	++	−	++	±	++	−	++	+ (18%)	++	+ (rare)	+	−	+	−	+	−

No reactivity was detected with 2BF11, N3A4 and HJ11.

2BD11 gave ± staining of Daudi and Raji fixed preparations.

N3C3 gave ± staining of SMS-SB, EB4 and Raji (rare) and + staining of 10% of Ed-1 fixed preparations.

B-LCL = B lymphoblastoid cell lines.

Two tests were performed for binding of MoAb to cell line cells: Sm = surface membrane binding to viable cells; C = binding to cells on a Cytospin slide preparation fixed with acetone.

All tests were performed by indirect immunofluorescence.

Table 4. Features of some CD-defined B cells MoAb reactive with FDC in normal tissue

Name of clone	Mol. Wt. of antigen	CD no. & specificity	FDC staining pattern Tonsil	NHL LN	B cells in tonsil sections	Cell line reactivity																			
						Pre-B		E B 4		Raji		Daudi		Ed-1		ARH-77		WM-1		8226		HSB-2		MOLT4		HL60	
						C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm	C	Sm
B4	P95	CD19	Total	−	+	−	++	−	+	−	++	−	++	−	++	−	++	−	++	−	++	−	−	−	−	−	−
BL13	P140	CD21 limited B	Total	Total	+	−	−	−	−	−	++	−	+	−	+	−	++	−	++	−	+	−	−	−	−	−	−
MHM6	P45	CD23 limited B	Sub-set	Sub-set	−	−	−	−	−	−	+	−	−	++	+	++	++	++	+	+	rare ++	−	−	−	−	−	−

NHL = non-Hodgkin's lymphoma; LN = lymph node; CD = cluster designation.

Two tests were performed for binding of MoAb to cell line cells: Sm = surface membrane binding to viable cells; C = binding to cells on a Cytospin slide preparation fixed with acetone.

All tests were performed by indirect immunofluorescence.

Two of the antibodies stained macrophages in tissue sections and monocytes in peripheral blood; of the seven antibodies showing some reactivity with lymphocytes in sections, only one (8EB1) gave bright staining of blood lymphocytes. These cells were shown by double immunofluorescence to coincide with the B lymphocytes identified by a B cell specific antibody (WR17)—but not with OKT3-positive cells. Other features of the MoAb summarized in the last column of Table 2 could be demonstrated with culture supernates of hybridomas as well as ascitic fluid and could therefore be attributed to the MoAb itself rather than background antibodies present in the ascitic fluid.

Many new antigens appear on lymphocytes following mitotic activation. Many of these antigens are particularly strongly expressed by continuously growing cell line cells. When tested against a large panel of haemopoietic cell line cells, MoAb of the B cell clusters CD19, CD21 and CD23 showed distinctive patterns of reactivity (Table 4). Some antigens are more readily demonstrable on viable cells, others on acetone-fixed cells. Three of the new MoAb (2BF11, N3A4 and HJ11) did not bind to cells of any of the lines tested (Table 3). Two MoAb (2BD11 and N3C3) detected antigens of very restricted expression across the panel. The remaining antibodies gave broad but distinctive patterns of binding confined in two cases (NC21 and 8EB1) to B cell lines. None of the MoAb gave patterns identical to those of CD19, CD21 or CD23 antibodies which are FDC-reactive.

The MoAb were also tested on sections of lymph nodes from four cases of low grade non-Hodgkin's lymphoma (centroblastic-centrocytic type) and the results are shown in Table 2. In sections of all four lymphomas the FDC were clearly stained by the reference antibodies R4/23 and BL13. All ten of the new collection of MoAb also stained FDC in sections of three of the four lymphomas but only 2BF11 gave unequivocal staining of FDC in the lymph node of case no. 3 (Table 2). Assessment of FDC staining was difficult with those MoAb which stained lymphocytes (HJ7 and 8EB1). Immunoglobulin was absent from the FDC in all four cases.

DISCUSSION

Antigens on FDC may be divided into three categories: (1) intrinsic antigens, found only on FDC; (2) intrinsic antigens shared with other cells or tissue constituents; (3) exogenous antigens passively acquired by FDC through surface receptors. A further consideration is that some intrinsic antigens may be concealed or induced as a result of cytophilic binding of immune complexes or from exposure to products of lymphocytes and other cells within the centre.

Only one of our MoAb (2BF11) showed exclusive FDC specificity when tested on histological sections and on blood leucocytes and cells of haemopoietic cell lines. It produced completely coincident staining when paired with R4/23. A second MoAb (N3A4) bound to FDC and no other cells but gave strong staining of connective tissue fibres in splenic trabeculae, blood vessel adventitia and other sites. One MoAb (HJ11) stained blood monocytes and tissue macrophages as well as FDC. It is of interest that both monocyte-binding antibodies reported by Ugolini *et al.* (1980) gave prominent staining of FDC and the FDC-reactive MoAb described by Murray *et al.* (1985) also stained monocytes. An unusual cross-reactivity was found with MoAb N3C3 which bound to FDC, to dendritic cells associated with tonsillar crypt epithelium and to dendritic cells in the outer cortex of fetal thymus. One MoAb (NC21) which stained FDC also stained blood platelets and endothelium and some macrophages. This agrees with a recent report of an epitope common to these three tissue components (Hogg *et al.*, 1984a). As expected, the MoAb E11 (Hogg *et al.*, 1984b), which is specific for the C3b receptor (CR1), bound strongly to FDC. The strong binding of BL-13 indicates that FDC express C3d as well as C3b receptors. FDC isolated from human tonsil have been shown to display Fc and C3 receptors (Heinen *et al.*, 1985).

FDC in three of the four follicle centre cell lymphomas were stained by the MoAb tested. However, in one of these lymphomas the FDC showed little or no reactivity with many of the test antibodies. The antibody 8EB1, which detects a B cell antigen, generally showed poor reactivity against FDC in neoplastic tissues.

In extension of our results with the B cell collection of MAbs of the Second International HLDA Workshop (when 13 out of 52 cross-reacted with FDC; Johnson *et al.*, 1985) we have found that 7 MoAb of a batch of 10 selected for FDC reactivity also detected antigens on cells of B lineage.

However, no other MoAb giving the restricted pattern of FDC staining previously described for three MoAb of the p45, CD23 cluster were encountered (Table 4).

The follicular localization of B cells in areas of lymphoid tissue where FDC are present suggests that FDC provide a micro-environment attractive to B cells. The marked proliferative activity of cells of B lineage within secondary follicles is driven by the antigen component of immune complexes bound to the surface of FDC; products of T lymphocytes and macrophages play a supplementary role. There is evidence that the appearance of immune complexes on the surface of FDC in germinal centres is dependent upon active transport of complexes to the follicle by B cells (Brown *et al.*, 1973; Gray *et al.*, 1984; Humphrey *et al.*, 1984). The mechanism by which the complexes are transferred from lymphocytes to FDC within the follicle is unknown, but it could well involve direct cell-cell transfer via complement and Fc receptors present on both cell types. This cell-cell interaction may account for the unexpectedly high incidence of cross-reactivity of anti-B cell MoAb with a totally unrelated cell type which has been shown not to be of bone marrow derivation (Humphrey *et al.*, 1984). Not all B cell surface antigens, however, are equally well expressed on FDC. For example, antigens associated with the CD21 and CD23 clusters are well expressed, the CD1 moderately and more variably and CD20 and CD22 antigens weakly or not at all.

One explanation of these results might be that surface structures associated with the binding of complexes to B cells are shed off with the complexes onto FDC when the two cell types adhere. A similar phenomenon may account for the presence of some macrophage antigens on FDC. Absence of antigen transfer in the abnormal germinal centres of follicle centre cell lymphomas could also account for their different antigen profile. However, the presence of immune complexes on the surface of FDC may modulate their display of surface antigens directly and thus account for antigenic differences between FDC in normal tissue and lymphomas since immune complexes are absent from FDC of neoplastic tissue.

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