ISOLATION AND CHARACTERIZATION OF HUMAN TONSIL DENDRITIC CELLS

By DEREK N. J. HART and JUDITH L. McKENZIE

From the Department of Haematology, Christchurch Hospital, Private Bag, Christchurch, New Zealand

Presentation of antigen in a suitable form for T cell recognition is required for the initiation of a primary immune response. Dendritic cells (DC)[1] purified from the lymphoid tissues of mice and rats have been identified as distinctive leukocytes (1) that have a remarkable ability to stimulate allogeneic T cells in mixed lymphocyte reactions (2) and also to present antigen effectively for primary immune responses (3). The Langerhans' cell in skin (4) and the interdigitating cell (5) found in the T cell areas of lymph node and tonsil are also considered to be members of a DC lineage with specialized antigen-presenting properties (1). Cells with similar dendritic morphology, which also stain strongly for class II MHC antigens, have been identified in non-lymphoid tissues (6) and act as a potent stimulus to rejection of organ allografts (7).

In man, DC have been described in blood (8, 9) and in the interstitium of non-lymphoid organs (10). As in rodents, human DC stimulate allogeneic T cells (8) and are capable of initiating primary immune responses (11). The exact relationship of DC, particularly the Langerhans' cell, to cells of the monocyte/macrophage lineage has not been fully resolved. In an attempt to isolate human DC without contamination by monocytes, we have purified DC from tonsils. These cells, which may represent the interdigitating cells seen in tissue sections, were shown to have a distinctive morphology similar to that described for mouse lymphoid DC. This DC population accounted for the ability of tonsil cells to stimulate T lymphocytes in autologous and allogeneic MLR. An extensive phenotypic analysis, which included numerous anti-macrophage mAbs, clearly identified tonsil DC as belonging to a distinct haemopoietic cell lineage.

Materials and Methods

mAbs. The majority of antibodies used in this study were obtained through the Third Leucocyte Differentiation Antigen Workshop (12). Other antibodies were purchased, or were gifts of colleagues (see Results and Tables I, II, and III for details). Positive control staining for all antibodies was obtained either as part of our contribution to the Workshop or by the use of an appropriate positive cell population.

This work was supported by a grant from the Medical Research Council of New Zealand, assisted in part by grants from the Cancer Society of New Zealand and the Canterbury Medical Research Foundation. D. N. J. Hart is in receipt of a Robert McClelland Fellowship.

[1] *Abbreviations used in this paper:* DC, dendritic cells; FH, Ficoll-Hypaque; HD, high density; LFA, leukocyte function-associated antigen; LD, low density; $SRBC_N$, neuraminidase-treated SRBC.

Cells. Tonsils from patients undergoing nonurgent tonsillectomies were collected in sterile saline within 30 min of removal. A single cell suspension was prepared by chopping the tissue finely and passing this material through a sieve. Viable mononuclear cells were obtained by centrifugation over a Ficoll-Hypaque (FH) 1.077 density gradient (>90% assessed by trypan blue exclusion). This cell suspension was depleted of T cells by rosetting with neuraminidase-treated SRBC ($SRBC_N$) for 1.5 h at 4°C. After centrifugation over an FH gradient, the T cell-depleted ($SRBC_N^-$) fraction was harvested from the FH interface. The T cell ($SRBC_N^+$) pellet was also recovered, and the SRBC were removed by hypotonic lysis with NH_4Cl lysis buffer.

The $SRBC_N^-$ fraction was resuspended in RPMI 1640 medium containing 10% FCS (Gibco, Auckland, N.Z.), and the cells were cultured in 100-mm plastic petri dishes (Nunc, Kamstrup, Denmark) for 1-2 d. After culture, the cells were resuspended in 5 ml BSA, d = 1.082 (Armour Pharmaceutical Co., Kankakee, IL), and overlayed with 2 ml BSA, d = 1.065, and 2 ml RPMI 1640. The gradients were centrifuged at 1,200 g for 15 min at 4°C, and the low density (LD), and high density (HD) cells were harvested. For final purification of DC, the LD cells were labeled with a mixture of B1 (CD20), BA1 (CD24), OKT3 (CD3), and 63D3 antibodies, followed by incubation with fluoresceinated goat anti-mouse Ig (Tago Inc., Burlingame, CA). Immunofluorescence double labeling was performed on a sample from initial preparation at this stage, using either a rhodamine- (CMRF-20) or phycoerythrin-conjugated (Becton Dickinson & Co., Mountain View, CA) mAb to HLA-DR, and viewed using a Leitz Ortholux microscope with appropriate filters. The labeled LD cells were then sorted into B1, BA1, OKT3, and 63D3 positive and negative populations using a FACS (IV; Becton Dickinson FACS Systems, Sunnyvale, CA). The FACS negative population was shown to contain the DC (see Results) and these DC preparations were used for the subsequent phenotypic analyses. In >100 preparations to date, only a few tonsils (mainly from adults) have failed to yield a FACS negative (DC) population, although, in many instances, the preparations at this stage contained <5% putative DC, and these were not sorted.

Mixed Lymphocyte Cultures. Graded doses of mitomycin C-treated (25 μg/ml, 30 min) stimulator cells from the various stages of the purification procedure were added to 2×10^5 responding T cells in 96-well microtest plates (16-3320, Nunc). The $SRBC_N^+$ lymphoid fraction obtained after rosetting was used as the responding cell population and were either unrelated to the donor (allogeneic MLR) or syngeneic to the donor (autologous MLR). Cultures were maintained in RPMI 1640 medium supplemented with 10% human AB serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Proliferation was measured with a pulse of 0.5 μCi of [^{3}H]thymidine (TRA.61, 5 Ci/mmol, Amersham Corp., UK) at 120-125 h.

Phagocytic Activity. Latex particles (1.0 μ, Sigma Chemical Co., St. Louis, MO) were opsonized in human AB serum and incubated with DC for 20 min at 37°C.

Phenotypic Analysis. Purified DC were analyzed for surface antigen expression using an extensive panel of antibodies and a sensitive double layer immunoperoxidase technique as described previously (13). Staining reactions were graded as definitely positive (+, ++, or +++, according to the intensity of membrane staining) or negative (−) compared with the control, and uncertain weak staining was recorded as ± (see Tables in Results). For intracytoplasmic staining, cytocentrifuge preparations of cells were fixed in methanol for 30 min at 4°C, before application of the appropriate antibody. This was followed by an incubation with a fluorescein-conjugated anti-Ig reagent, and slides were read using a Leitz Ortholux microscope with UV light source.

Cytochemical Staining. Nonspecific esterase staining was performed using α naphthyl-butyrate as a substrate. Peroxidase and acid phosphatase staining utilized standard cytochemical techniques.

Morphological Analysis. For analysis by light microscopy, cytocentrifuge preparations of purified cells were fixed and stained using May-Grunwald-Giemsa stain. Transmission electron microscopy was performed on cells after fixation in 2.5% glutaraldehyde and post-fixation in 1% osmium tetroxide. The samples were then stained with uranyl acetate, dehydrated, and embedded in Spurr's resin.

Results

Purification of DC. The majority (consistently >95%) of cells obtained in a single cell tonsil suspension were CD3 or CD19 positive by FACS analysis, i.e., T and B lymphocytes. Very few 63D3 positive cells (monocytes/macrophages) or CD16 positive (NK) cells were present. After removal of T cells by rosetting and density gradient centrifugation of the cultured cells, the LD cells obtained were examined by phase contrast. Most of the cells were large, and many had an irregular profusion of membrane processes as described for DC. However, although FACS analysis revealed that all of these cells stained strongly for HLA-DR antigens, the majority also stained with CD19, CD20, and anti-Ig reagents, identifying them as B cells. Further fractionation of the cells was then undertaken by labeling the cells with a mixture of antibodies directed at T cell, B cell, and monocyte markers, and then separating positive and negative cells (Fig. 1). In successful preparations, this procedure revealed a minor LD population, generally 5–20% of the cells analyzed, which were CD3, CD20, CD24, and 63D3 negative, but stained strongly for HLA-DR antigens. This cell population containing putative DC (~0.1% of the starting tonsil cell suspension) was then subjected to further analysis.

Mixed Lymphocyte Culture. As one of the most important characteristics of DC is their capacity to stimulate strongly in MLR, each fraction obtained during our purification procedure was tested for its capacity to stimulate purified T cells. In allogeneic MLR (Fig. 2 *A*), the stimulatory capacity was identified in the $SRBC_N^-$ fraction, with the $SRBC_N^+$ fraction containing little or no stimulatory activity. After culture and density gradient centrifugation, the LD cellular fraction stimulated a strong T cell-proliferative response, accounting for virtually all the stimulatory ca-

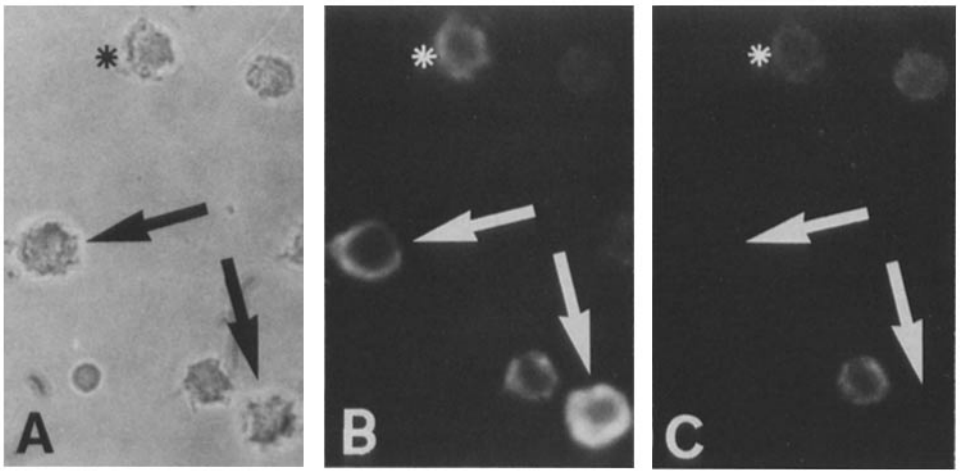

FIGURE 1. Double immunofluorescence staining of tonsil DC before further FACS IV purification. Rhodamine-conjugated anti-HLA-DR mAb (CMRF-20) was used to stain cells directly, and the mAb mixture (B1, BA1, OKT3, and 63D3) stained indirectly with fluoresceinated goat anti-mouse Ig. (*A*) Phase contrast view, putative DC (*arrows*); (*B*) anti-HLA-DR stains DC strongly (*arrows*); (*C*) DC are negative (*arrows*) with the mAb mixture, but note the larger strongly HLA-DR^+ cell (probable B cell) which is stained (*). ×40.

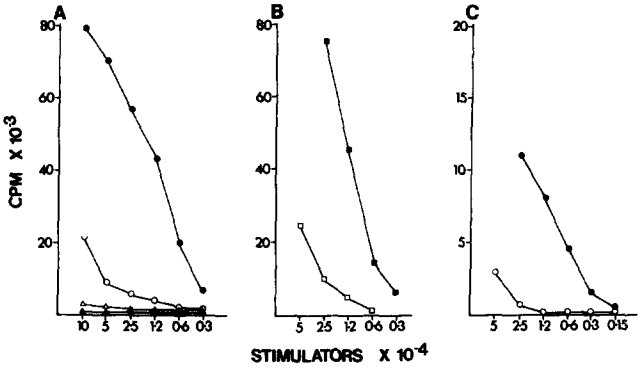

FIGURE 2. Stimulatory capacity of isolated DC in MLR. Graded doses of E_N^+ (Δ), E_N^- (○), LD (●), HD (▲) tonsil cells were used to stimulate 2 × 10^5 allogeneic responding T cells (*A*). After FACS sorting FACS-positive (□) or FACS-negative (■) tonsil cell stimulators were likewise added to allogeneic T cells (*B*). In *C*, autologous T cell responders were used. Results are expressed as cpm of tritiated thymidine incorporated.

pacity in isolated tonsil cells. The HD fraction, on the other hand, failed to stimulate allogeneic T cells. The major population of LD cells were B lymphocytes, with a minor population of putative DC. After labeling with CD20, CD24, CD3, and 63D3 antibodies, the positive and negative cell populations isolated by the FACS were tested for their ability to stimulate in MLR. The results of such an experiment comparing the stimulatory activity of the FACS positive cells (B cells) with the FACS negative cells (DC) is shown in Fig. 2 *B*. Although the B cell fraction was able to stimulate to a limited extent, the FACS negative (DC population) had by far the most potent stimulatory capacity. The LD DC-enriched fraction was also considerably more effective at stimulating T cells in an autologous MLR (Fig. 2 *C*).

Morphology of DC. FACS-purified DC preparations appeared relatively homogeneous (Fig. 3 *A* and *B*). The cells were generally considerably larger in size than unstimulated lymphocytes (15–20 μm) and were of variable shape. There was some variation in the number of cell membrane protrusions, and a few cells appeared rounded. Staining the cell membrane with anti-HLA class II reagents was required to demonstrate the full extent of the dendritic processes (Fig. 3 *C*), and some of these were particularly long and delicate (Fig. 3 *D*). The cytoplasm was lightly basophilic but less intensely so in the perinuclear region, suggesting a prominent Golgi apparatus. In some preparations, small vacuoles were noted, but no granules were seen. The nucleus was large and usually indented with open chromatin and a visible nucleolus. At an EM level, the cytoplasm was shown to contain numerous mitochondria, free ribosomes, little rough endoplasmic reticulum, and a large Golgi apparatus. No lysozomal structures were identified.

Phagocytic Activity and Cytochemical Staining. Purified DC did not ingest opsonized latex particles, whereas control preparations of monocytes and neutrophils did. Cytochemical staining of DC was compared with monocyte/macrophage control reactions. Some DC were very weakly positive for nonspecific esterase with a very occasional moderately positive cell noted, but these stained much less intensely than $63D3^+$ cells. The DC were variably positive for acid phosphatase (tartrate sensitive), and this staining was diffuse and confined mainly to the perinuclear region (no granules were noted) (Fig. 3 *E*). Peroxidase, PAS, Oil Red O, and β-glucuronidase staining was consistently negative.

Phenotypic Analysis with Non-lineage-specific Antibodies. The DC stained positively

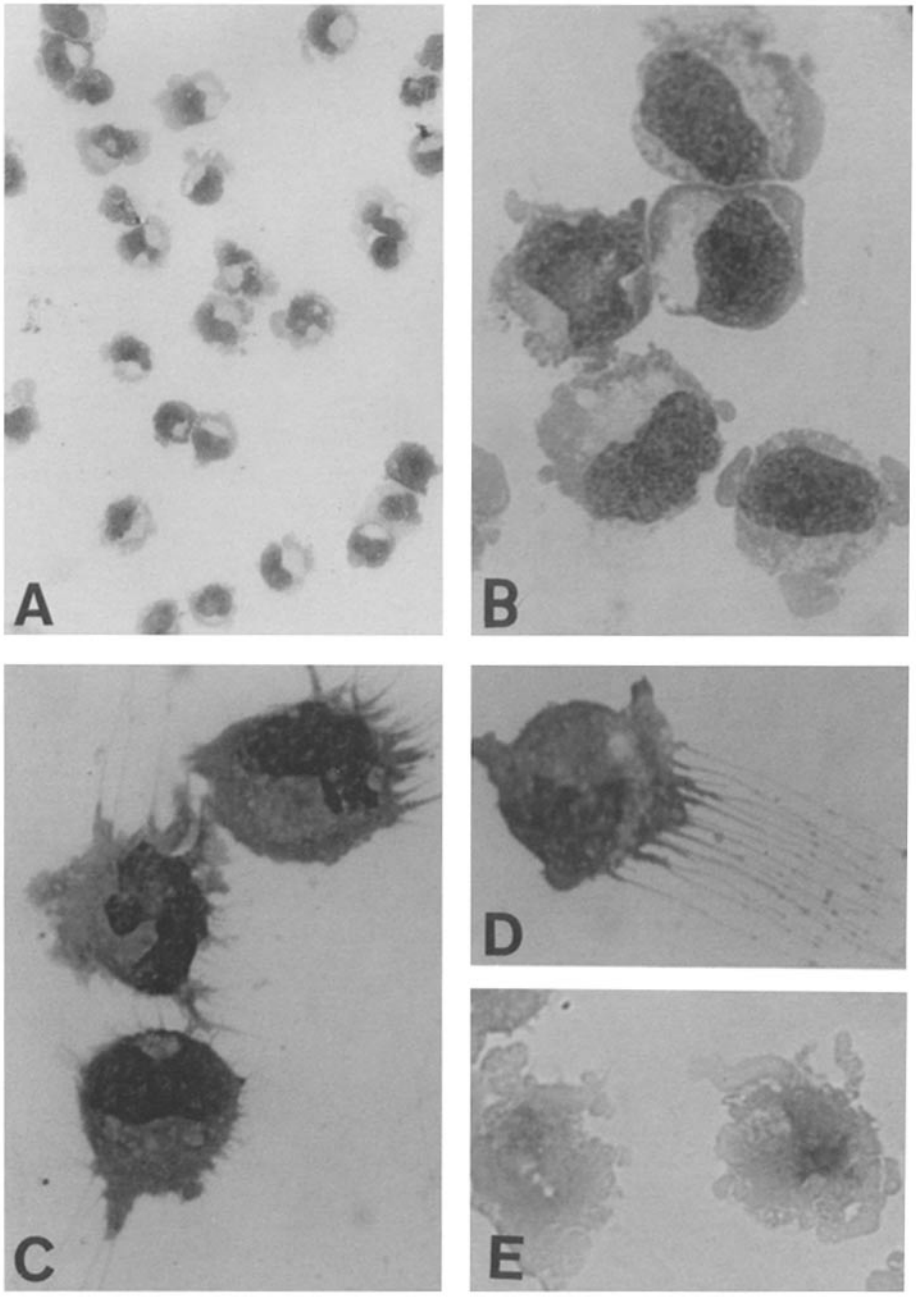

FIGURE 3. Human tonsil DC. The appearance of MGG-stained FACS-purified cells is shown in *A* (×7.5) and *B* (×63). (*C*) Immunoperoxidase staining with anti-HLA-DR reagent emphasises the dendritic processes that are particularly long and delicate in some cells (*D*). Cytochemical staining with acid phosphastase (*E*) reveals positive staining adjacent to the nucleus. C, D, and E, ×63.

TABLE I

Reactivity of Non-lineage-specific Antibodies with Isolated DC

Cluster of differentiation/ specificity	mAb	Reactivity with DC	Source or reference
HLA-DP	HLA-DP	+ + +	BD*
-DQ	Leu-10	+ + +	BD
-DR	HLA-DR, DA2	+ + +	BD, 12
-DQ + DR	CMRF-20	+ + +	This laboratory
HLA-ABC	CMRF-6	+	This laboratory
CD11a	MHM24, CIMT	+	12
CD11b	OKM1, 44, MO1	–	12
CD11c	KB23, 3.9, Ki-M1, Bu15	–	12
CD18	MHM23, 60.3	+	12
CD45	CMRF-12, CMRF-26	+ +	14
CD45R	CMRF-11	+	14
	F8.11.13	+	12

* Becton Dickinson & Co., Mountain View, CA.

with several non-lineage-specific antibodies, as detailed in Table I. Intense staining was seen with a variety of antibodies to MHC class II antigens, including antibodies specific for HLA-DP, -DQ, and -DR antigens. Although quantitative studies were not performed, the intensity of staining seen with these three antibodies suggested that the DC expressed similar amounts of the three MHC class II products. In addition, the cells expressed class I MHC antigens and reacted with several antibodies to the leucocyte common antigen, including antibodies to different epitopes on the restricted and unrestricted determinants (CD45 and CD45R).

Several antibodies to the leucocyte function associated (LFA) antigens were tested. The DC were positive for CD11a antibodies, recognizing the α chain of LFA-1, and also, as expected, CD18 antibodies, which recognized the β chain of the LFA family. The CD11b and CD11c antibodies, which react with the α chains of other LFA family molecules, with a somewhat more restricted tissue distribution, were consistently negative.

Phenotypic Analysis with T and B Lymphocyte Antibodies. The panel of antibodies against T and B lymphocyte antigens that were tested on isolated DC, and the results obtained, are shown in Table II. DC lacked virtually all of the well-defined T and B cell antigens, including those detected by CD1 antibodies, which are known to be markers for epidermal Langerhans' cells. The only T cell antibody that gave significant staining was the CD4 antibody Leu-3, which gave variable weak labeling of some DC. The other CD4 antibody used, OKT4, was consistently negative.

Similarly, very few B cell antibodies stained isolated DC. However, antibodies from the two most recently defined B cell antibody clusters, CD39 and CDw40, were both positive, as were two unclustered antibodies, 2-7 and 7F7 (12). Examples of DC stained using these antibodies with the immunoperoxidase technique are shown in Fig. 4. The CD39 reagents showed a gradation in staining intensity with some DC staining only weakly (Fig. 4C). Intracytoplasmic staining failed to reveal cytoplasmic IgM or IgG, and the nuclei failed to stain for terminal deoxynucleotide transferase (TdT; Bethesda Research Laboratories, Gaithersburg, MD).

TABLE II
Reactivity of T and B Cell Antibodies with Isolated DC

Cluster of differentiation/ specificity	mAb	Reactivity with DC	Source* or reference
CD1 a	NA1/34	–	15
b	WM-25	–	12
c	7C6/162/3B10	–	12
CD2	OKT11	–	ATCC
CD3	OKT3	–	ATCC
CD4	OKT4	–	ATCC
	Leu 3	+	BD
CD5	UCHT1	–	16
CD7	WT1	–	17
	HuLyM2	–	18
CD8	OKT8	–	ATCC
CD9	FMC8	–	19
CD10	AL2	–	20
CD19	B4	–	C
CD20	B1	–	C
CD21	B2	–	C
	BL13/10B1	–	12
CD22	HD-39	–	12
CD23	PL-13	–	12
CD24	BA1	–	H
CD37	BL14/10B1a	–	12
CD39	G28-10, G28-8	+	12
CDw40	G28-5	+	12
B cell subset	FMC7	–	21
B cells	FMC1	–	21
Restricted B + IDC	2-7	+ +	12
Restricted B + IDC	7F7	+	12
Plasma cells	PCA1	–	C
Surface Ig	SmIg	–	This laboratory

* ATCC, American Type Culture Collection, Rockville, MD; BD, Becton Dickinson & Co., Mountain View, CA; C, Coulter Immunology, Hialeah, FL; H, Hybritech Inc., La Jolla, CA.

Phenotypic Analysis with Antibodies to Myeloid Antigens. Results obtained with a range of myeloid antibodies, including the myeloid panel from the Third Leucocyte Antigen Workshop, are shown in Table III.

Almost all of the well-defined myeloid antibody clusters, in particular the macrophage reactive antibodies, failed to stain isolated tonsil DC. Furthermore the cells expressed no Fc receptors, as defined by antibody 32 (FcRI) or CDw32(FcRII) and CD16 (FcR_{lo}) antibodies. DC were also negative with CD35, CD21, and CD11b antibodies, which recognize complement receptors CR1 (C3b), CRII (C3d), and CRIII (C3bi), respectively.

Some weak positive staining was obtained with a few antibodies from the myeloid panel, but none of the antibodies involved were truly myeloid cell specific. Some, but not all, of the CD13 antibodies stained DC weakly, but this positivity was found to vary from preparation to preparation, and four of the antibodies in this cluster

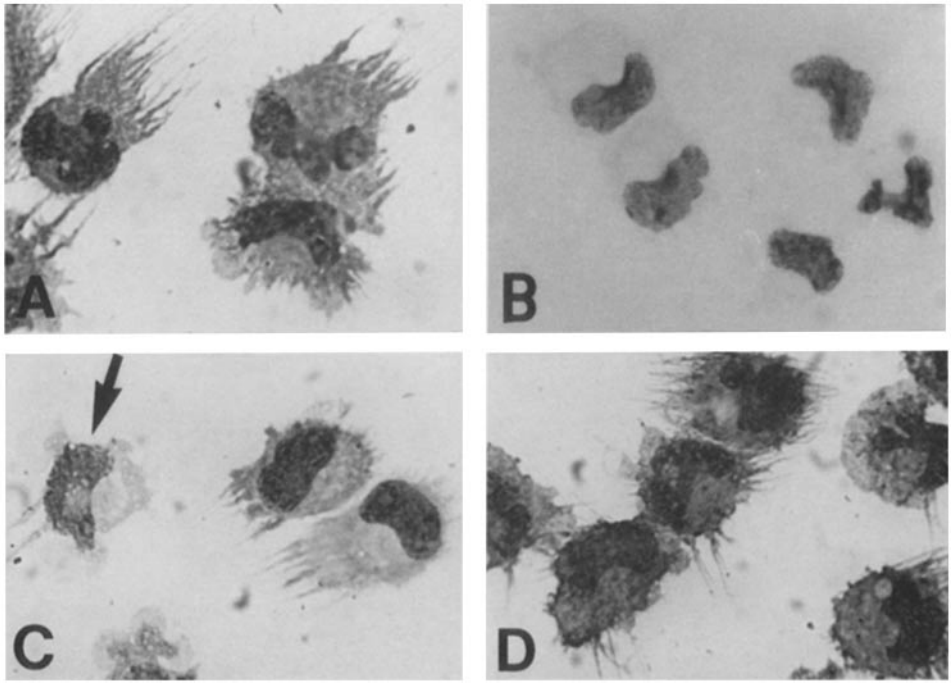

FIGURE 4. Immunoperoxidase staining of purified tonsil DC using a sensitive double-layer immunoperoxidase technique. (*A*) mAb G28.5 (CDw40) is positive (+). (*B*) B4 (CD19) is negative (−). (*C*) mAb G28.8 (CD39) is positive (+) but some cells (*arrow*) are weakly stained. (*D*) The unclustered antibody 2-7 stains DC strongly (++). ×63.

were consistently negative. The CD13 antibodies, which recognize a carbohydrate residue, are known to stain collagen, renal glomeruli and proximal tubules, osteoclasts, and liver-bile canuliculi (12), in addition to monocytes and granulocytes.

Both antibodies from the CD31 cluster, which detect an antigen found not only on monocytes, granulocytes, platelets, and some T cells, but also on epithelium and endothelium (12), stained DC weakly. One antibody, clustered as CD15c, always gave a strong positive result, but in our hands, this antibody was unusual in that it reacted with most haemopoietic (and many non-haemopoietic) cells tested.

Some additional antibodies that were not assigned a CD number at the Third Leucocyte Differentiation Antigen Workshop, namely those in Groups 13, 14, and 15, did show some variable positivity. The staining pattern of these groups of antibodies is known to be widespread and includes endothelium, epithelium, muscle, monocytes, platelets, and hepatocytes (12).

Staining of DC with Antibodies to Activation Markers and Other Antigens. A variety of mAbs to activation antigens were also tested; while the CD30 antibody RSC-1 (Dako Corp., Santa Barbara, CA) and antibodies to the transferrin receptor, OKT9, (American Type Culture Collection (ATCC), Rockville, MD), and CMRF-2 (this laboratory) were negative, several antibodies did give a positive result. The antibodies 4F2 (ATCC) and MMA (ATCC), which are known to react with activated cells, stained DC in a reproducible fashion.

TABLE III
Reactivity of Myeloid Cell Antibodies with Isolated DC

Cluster of differentiation/ specificity	mAb	Reactivity with DC	Source* or reference
CD13	My7, WM15, MoU48, MoU28,	-	12
	SJ-IDI, CLB-mon-gran/2, MCS2	±	12
CD14 a	FMC 17, UCHM1	-	12
CD14 b	My4, MEM15, MEM18, Cris6, CLB-mon/1	-	
	MA5, TM3, TM2, MoP9, FMC32, MoS1,	-	12
	RM050, 3C10	-	
CD15 a	IGI0, VIMD5, MZ17, Thy-22	-	12
	CMRF-7	-	13
CD15 b	GF.26.7.3	-	12
CD15 c	SYB-8	-	12
	8-27-F6	+ +	12
CD16	Leu11a	-	BD
	BW209/2, CLB/FcR gran 1, MG38	-	12
CD17	T5A7	-	12
CD31	SG134, TM3	±	12
CD32	CIKM5, 2E1	-	12
	IV.3	-	22
CD33	My9, LIB2	-	12
CD34	B1-3C5, My10	-	12
CD35	C3b	-	Dako
	E11, J3B11, J3D3	-	12
CD36	5F1	-	12
W/S group 9	88H4, G10F5	-	12
W/S group 10	20E7, SG133, TE-3A-2C4	-	12
W/S group 11	VIM8	-	12
W/S group 12A	24, Y2/131, KiM6, Y-1/82A, EMB11	-	12
W/S group 12B	10.1, HL21, Ki-M8, Ki-M7	-	12
W/S group 13	MAX-1	-	12
	MAX-24	±	12
W/S group 14	8FB	±	12
W/S group 15	82H1, MOF11	±	12
W/S group 16	M67	-	12
Follicular DC	DRC1	-	Dako
Neutrophils	LeuM4	-	BD
Monocytes	LeuM3	-	BD
Monocytes	63D3	-	23
FcRI	32	-	22
Erythrocytes	CMRF-4, CMRF-10, CMRF-17	-	24

* BD, Becton Dickinson & Co.; Dako, Dako Corp., Santa Barbara, CA.

In addition to the antibodies listed in the tables, a variety of other antibodies were also tested for their reactivity with DC. An antibody specific for follicular DC, DRC-1 (Dako Corp.) was consistently negative on our DC preparations, as were the NK cell markers NKH-1 (ATCC) and NKH1 (Coulter Electronics Inc., Hialeah, FL). Several antibodies that recognize erythrocyte glycoproteins (24) and an antibody against human Thy-1 (25) were also negative.

Staining DC with Antibodies to IL-1. Cytoplasmic staining with the mAbs 13D12

and 3A4 to IL-1-α and IL-1-β, respectively, was performed (26). Strong positive intracytoplasmic staining of control LPS-stimulated monocytes was obtained, but no staining of tonsil DC was seen.

Discussion

The purification procedure described here isolated an LD population of cells from human tonsils that were strongly HLA class II positive, had the morphologic appearance of DC, and stimulated strongly in MLR. Several studies have taken advantage of the LD property of DC to aid in their purification, and murine DC can be obtained even more readily as a result of their partial adherence to plastic (1). The cells obtained from such procedures are often classified as DC on these criteria alone, but our data would suggest that in man the majority of the LD tonsil cells also express B cell markers. The fact that human B cells can undoubtedly assume a dendritic morphology in culture is not surprising in view of a recent report (27) drawing attention to the fact that peripheral blood B cells assumed a DC morphology after stimulation with the phorbol ester PMA. Clearly, this means that considerable care is required to characterize human DC preparations before studying their characteristics further. The contaminating B cell population in our preparations was found to stimulate weakly in MLR; in contrast, the minor population of cells that were negative for the CD24 and CD20 B cell markers were found to be the most potent stimulators in MLR. This population of cells was consistently identified in numerous tonsil preparations, but the numbers were small (0.1% of all tonsil cells). Some variation in numbers from different individuals is not unexpected in view of the differences in interstitial DC content previously noted in different rat strains (7). It is also possible that other variables, including the degree of tonsil inflammation, influenced yields.

The DC purified from tonsil have a morphological appearance that distinguishes them from macrophages. The ultrastructural features of these cells are similar to those described for murine DC (1), and no Birbeck granules typical of Langerhans' cells were seen. Of note, a prominent Golgi apparatus was identified, and this probably accounts for the acid phosphatase staining noted in these cells. Interestingly, we did note some minor vacuolation in these cells, and although the cells do not phagocytose latex particles, further investigation of their ability to take up antigen is clearly warranted. Since considerable preparation was required to purify the DC, it is possible that the minor degree of vacuolation is a degenerative change. Overall, the features are consistent with current knowledge of the structure of interdigitating cells in tissue sections (5).

It has not yet been possible to assign DC clearly to any of the known haemopoietic cell lineages (1, 28), although the Langerhans' cell in the skin expresses the CD1 antigen and has been suggested to have some relationship to the monocyte/macrophage lineage, because it has Fc and C3 receptors and phagocytic capability (4). Although some studies have suggested that the DC belongs to the monocyte/macrophage lineage (29, 30), our results do not support this hypothesis. The paucity of positive reactions obtained with monocyte/macrophage antibodies, the lack of Fc and complement receptors, and the weak positivity for myeloid related enzymes make it unlikely that the DC isolated from tonsil belong to this particular cell lineage.

It is also unlikely, given the results we obtained with antibodies to T cell, B cell, NK cell, and erythrocyte antigens, that the DC are related to any of these cell lineages.

The reaction of certain antibodies with the tonsil DC is worthy of comment. The weak reactivity of Leu-3 with these cells is consistent with its reported expression on interdigitating cells (29, 30) and Langerhans' cells (31), both of which are part of the DC series. Similarly the CDw40 antibody G28-5 and the antibodies 2-7 and 7F7 that stained tonsil DC have been reported to stain interdigitating cells (32). Other antibodies, such as those of the CD13 cluster that were positive, cannot be interpreted as indicating lineage specificity as they react with a wide range of different cell types (12). The CD39 antibodies, for instance, are positive on B cells, activated T cells, monocytes, macrophages, and blood vessel endothelium. Even so, it is noteworthy that some of these nonlineage specific antibodies also react with Langerhans' cells in the skin (33). Overall, it would seem then that the human DC stems from a unique differentiation pathway, which one could postulate may include Langerhans' cells, lymphoid DC, interdigitating cells, and interstitial DC. Indeed, an extensive set of morphological, phenotypic, and functional comparisons of murine epidermal Langerhans' cells with spleen DC has recently been described by Schuler et al. (34). These studies showed that isolated murine Langerhans' cells, which were NSE, ATPase, F4/80 (anti-macrophage), and 2.4G2 (anti-F_c receptor) positive, lost these characteristics and became better stimulators of T cells in culture, i.e., similar to spleen DC, and the authors suggested that Langerhans' cells were DC precursors. The modulation of F_c receptors is particularly interesting, as we have noted that human Langerhans' cells in skin, like tonsil DC, fail to stain with mAbs to F_cR1, F_cRII, and F_cRIII.

The DC we obtained did express HLA.ABC and HLA.DP, DQ, and DR antigens, as well as one of the leucocyte function-associated antigens (LFA-1). It is remarkable how few other molecules have so far been detected on the cell surface of DC, especially when one considers how many different antigens are detected on other cell types. To date, cell membrane analysis has failed to reveal a specific marker for human DC analogous to the antigen on mouse DC (35). We have noticed that anti-HLA-DQ reagents do stain DC somewhat more selectively than anti-HLA-DR reagents, thereby resulting in apparent specificity for DC that express HLA-DQ in high density relative to other cell types. This may explain the results reported using the antibody RFD-1 (36).

The expression of certain potential activation antigens (MMA, 4F2) on the isolated DC was of interest. It is uncertain whether this was induced during the culture period, as described for inflammatory macrophages and IL-2-R (37), or whether resting DC express these antigens. Initial studies on the expression of IL-2-R on isolated DC have given conflicting results, although staining skin (Davis et al., submitted for publication) and liver (Prickett et al., submitted for publication) sections fails to reveal IL-2-R on DC. The recent description of high- and low-affinity receptors for IL-2 (38) makes it appropriate for us to investigate the effect of IL-2 on DC function.

Although many other MHC class II positive cell types are capable of presenting antigen in a secondary immune response, the DC family seems to be unique in its capacity to stimulate a primary response (39). The reasons for this are unclear. Although this may be a consequence of high density class II MHC antigen expression in combination with multiple membrane contacts with the interacting T cells, it is

possible that DC may possess lineage specific, as yet undetected membrane molecules or lymphokines, which are responsible for this unique function. Our inability to demonstrate IL-1α or IL-1β in these cells increases our enthusiasm in the search for such molecules. However, we wish to emphasise that an irregular cell membrane and MHC class II antigen positivity does not necessarily identify a DC, and we would stress that progress in analyzing human DC will only result from investigating highly purified populations shown on the basis of T cell stimulation and surface marker studies to be homogeneous cell populations.

Summary

Human dendritic cells were isolated from tonsils by density gradient separation followed by FACS IV sorting with mAbs to remove contaminating cell populations. The resulting dendritic cell population consisted of large cells with plentiful basophilic cytoplasm, lacking in granules but containing a prominent Golgi apparatus and numerous mitochondria. The cell membrane was irregular, and marked cell protrusions were obvious when stained with anti-HLA class II reagents. Their nuclei were irregular and often indented with a visible nucleolus. These cells were not phagocytic and stimulated autologous and allogeneic lymphocytes more effectively than other tonsil cell types in MLR. Phenotypic analysis of these cells confirmed that they expressed the leucocyte common antigen and stained strongly for HLA-class II antigens. Tonsil dendritic cells also coexpressed the LFA-1α and LFA-1β chains but did not stain with a wide variety of anti-monocyte or anti-macrophage antibodies. The cells also lacked Fc and complement receptors and failed to stain with CD1 antibodes. Extensive testing with mAbs revealed only a few positive reactions, and these were consistent with reports of these antibodies staining interdigitating cells in tissue sections. This established that tonsil dendritic cells belong to the unique haemopoietic cell lineage of dendritic cells. No cytoplasmic staining of IL-1α or IL-1β was demonstrated, although these lymphokines were readily detected in activated monocytes.

The authors thank Mr. Tim Prickett for his considerable help with the DC isolation procedures; Mr. Tony Day for assisting with the electron microscopy; and Ms. Gail James for the typing of this manuscript. We are most grateful to Associate Professor M. E. J. Beard for his advice and encouragement and also to our colleagues who assisted by providing the clinical material.

Received for publication 7 January 1988 and in revised form 21 March 1988.

References

1. Steinman, R. M., and M. C. Nussenzweig. 1980. Dendritic cells: features and functions. *Immunol. Rev.* 53:127.
2. Steinman, R. M., B. Gutchinov, M. D. Witmer, and M. C. Nussenzweig. 1983. Dendritic cells are the principal stimulators of the primary mixed leucocyte reaction in mice. *J. Exp. Med.* 147:613.
3. Inaba, K., M. D. Witmer, and R. M. Steinman. 1984. Clustering of dendritic cells, helper T lymphocytes, and histocompatible B cells, during primary antibody responses in vitro. *J. Exp. Med.* 160:858.

4. Silberberg-Sinakin, I., R. L. Baer, and G. J. Thorbecke. 1978. Langerhans cells: a review of their nature with emphasis on their immunological functions. *Prog. Allergy.* 24:268.
5. Veerman, A. J. P., and W. Van Ewijk. 1974. White pulp compartments in the spleen of rats and mice. A light and EM study of lymphoid and non-lymphoid cells in T and B areas. *Cell Tissue Res.* 156:427.
6. Hart, D. N. J., and J. W. Fabre. 1981. Demonstration and characterisation of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. *J. Exp. Med.* 153:347.
7. McKenzie, J. L., M. E. J. Beard, and D. N. J. Hart. 1984. The effect of donor pretreatment on interstitial dendritic cell content and rat cardiac allograft survival. *Transplantation (Baltimore).* 38:371.
8. Kuntz Crow, M., and H. G. Kunkel. 1982. Human dendritic cells: major stimulators of the autologous and allogeneic mixed leucocyte reactions. *Clin. Exp. Immunol.* 49:338.
9. Van Voorhis, W. C., S. L. Hair, R. M. Steinman, and G. Kaplan. 1982. Human dendritic cells: enrichment and characterisation from peripheral blood. *J. Exp. Med.* 155:1172.
10. Daar, A., S. Fuggle, D. N. J. Hart, R. Dalchau, Z. Abdulaziz, J. W. Fabre, A. Ting, and P. J. Morris. 1983. Demonstration and phenotypic characterisation of HLA-DR-positive interstitial dendritic cells widely distributed in human connective tissues. *Transplant. Proc.* 15:311.
11. Inaba, K., R. M. Steinman, W. C. Van Voorhis, and S. Muramatsu. 1983. Dendritic cells are critical accessory cells for thymus-dependent antibody responses in mouse and in man. *Proc. Natl. Acad. Sci. USA.* 80:6041.
12. McMichael, A. J., P. C. L. Beverly, S. Cobbold, M. J. Crumpton, et al., editors. 1987. Leucocyte Typing III: White Cell Differentiation Antigens. Oxford University Press, Oxford. 1050 pp.
13. Davidson, S. E., J. L. McKenzie, M. E. J. Beard, and D. N. J. Hart. 1988. The tissue distribution of the 3α fucosyl-N-acetyl lactosamine determinant recognised by the monoclonal antibodies CMRF-7 and 27. *Pathology.* 20:24.
14. Starling, G. C., S. E. Davidson, J. L. McKenzie, and D. N. J. Hart. 1987. Inhibition of natural killer cell-mediated cytolysis with monoclonal antibodies to restricted and non-restricted epitopes of the leucocyte common antigen. *Immunology.* 61:351.
15. McMichael, A. J., J. R. Pilch, G. Galfre, D. M. Mason, J. W. Fabre, and C. Milstein. 1979. A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. *Eur. J. Immunol.* 9:205.
16. Beverly, P. C. L., and R. E. Callard. 1987. Distinctive functional characteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody. *Eur. J. Immunol.* 11:329.
17. Vodinelich, L., W. Tax Y. Bai, S. Pegrim, P. Capel, and M. F. Greaves. 1983. A monoclonal antibody (WT1) for detecting leukaemias of T cell precursors (T-ALL). *Blood.* 62:1108.
18. Thurlow, P. J., K. E. Lovering, and I. F. C. McKenzie. 1984. A monoclonal antibody detecting a new human T cell antigen, HuLy-M2. *Transplantation (Baltimore).* 38:143.
19. Brooks, D. A., J. Bradley, and H. Zola. 1982. A differentiation antigen expressed selectively by a proportion of human blood cells: detection with a monoclonal antibody. *Pathology.* 14:5.
20. Lebacq-Verheyden, A. M., A. M. Ravoet, H. Hazin, D. Sutherland, N. Tidman, and M. F. Greaves. 1983. Rat AL2, AL3, AL4 and AL5 monoclonal antibodies bind to the common acute lymphoblastic leukaemic antigen (CALLA gp100) *Int. J. Cancer.* 32:273.
21. Zola, H. 1987. The surface antigens of human B lymphocytes. *Immunol. Today.* 8:308.
22. Anderson, C. L., and R. J. Looney. 1986. Human leucocyte IgG Fc receptors. *Immunol. Today.* 7:264.

23. Ugolini, V., G. Nunez, R. G. Smith, P. Stastny, and J. D. Capra. 1980. Initial characterization of monoclonal antibodies against human monocytes. *PNAS.* 77:6764.
24. Anderson, S. E., J. L. McKenzie, K. McLoughlin, M. E. J. Beard, and D. N. J. Hart. 1986. The inheritance of abnormal sialoglycoproteins found in a Gerbich negative individual. *Pathology.* 18:407.
25. McKenzie, J. L., and J. W. Fabre. 1981. Human Thy-1: unusual localisation and possible functional significance in lymphoid tissue. *J. Immunol.* 126:483.
26. Conlon, P. J., K. H. Grabstein, A. Alpert, K. S. Prickett, T. P. Hopp, and S. Gillis. 1987. Localization of human mononuclear cell interleukin 1. *J. Immunol.* 139:98.
27. Corradi, M. P., D. F. Jelinek, J. E. Ramberg, and P. E. Lipsky. 1987. Development of a cell with dendritic morphology from a precursor of B lymphocyte lineage. *J. Immunol.* 138:2075.
28. Tew, J. G., G. J. Thorbecke, and R. M. Steinman. 1982. Dendritic cells in the immune response: characteristics and recommended nomenclature. *J. Reticuloendothel. Soc.* 31:371.
29. Wood, G. S., R. R. Turner, R. A. Shiurba, L. Eng, and R. A. Warnke. 1985. Human dendritic cells and macrophages. *Am. J. Pathol.* 119:73.
30. Hancock, W. W., and R. C. Atkins. 1984. Immunohistologic analysis of the cell surface antigens of human dendritic cells using monoclonal antibodies. *Transplant. Proc.* 16:963.
31. Wood, G. S., N. L. Warner, and R. A. Warnke. 1983. Anti-Leu 3/T4 antibodies react with cells of monocyte/macrophage and Langerhans lineage. *J. Immunol.* 131:212.
32. Ling, N. R., I. C. M. Maclennan, and D. Y. Mason. 1987. B-cell and plasma cell antigens: new and previously defined clusters. Leucocyte Typing III: White Cell Differentiation Antigens. Oxford University Press. 302–335.
33. McKenzie, J. L., G. F. Reid, M. E. J. Beard, and D. N. J. Hart. 1987. Reactivity of myeloid monoclonal antibodies with human dendritic cells. Leucocyte Typing III: White Cell Differentiation Antigens. Oxford University Press. 668–671.
34. Schuler, G., N. Romani, and R. M. Steinman. 1985. A comparison of murine epidermal Langerhans cells with spleen dendritic cells. *J. Invest. Dermatol.* 85:99s.
35. Nussenzweig, M. C., R. M. Steinman, M. D. Witmer, and B. Gutchinov. 1982. A monoclonal antibody specific for mouse dendritic cells. *Proc. Natl. Acad. Sci. USA.* 79:161.
36. Poulter, L. W., D. A. Campbell, C. Munro, and G. Janossy. 1986. Discrimination of human macrophages and dendritic cells by means of monoclonal antibodies. *Scand. J. Immunol.* 24:351.
37. Hancock, W. W., L. Kobzik, A. J. Colby, C. J. O'Hara, A. G. Cooper, and J. J. Godleski. 1986. Detection of Lymphokines and Lymphokine Receptors in Pulmonary Sarcoidosis. Immunohistologic Evidence that Inflammatory Macrophages Express Il-2 Receptors. *Am. J. Pathol.* 123:1.
38. Trowbridge, I. 1987. Interleukin-2 receptor proteins. *Nature (Lond.).* 327:461.
39. Inaba, K., and R. M. Steinman. 1984. Resting and sensitized T lymphocytes exhibit distinct stimulatory (antigen-presenting cell) requirements for growth and lymphokine release. *J. Exp. Med.* 160:1717.