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Class II antigens on dendritic cells from the synovial fluids of patients with inflammatory arthritis

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SUMMARY

Dendritic cells were enriched from synovial fluids (SF) of patients with inflammatory arthritis and studied by immunogold labelling and electron microscopy for expression of histocompatability antigens of the HLA-D locus. Dendritic cells from SF were larger than most of these from peripheral blood with a more extensive Golgi region and more lysosomes and microfilaments. Class II histocompatability antigens HLA-DR, -DP, -DQ and that labelled by the antibody RFDI were abundant on the dendritic cells. The macrophages in the enriched cells showed labelling for DR but little labelling with the other antibodies. DR, DP and RFDI were often concentrated at areas of contact between dendritic and other cells (other dendritic cells, macrophages or lymphocytes). On incubating labelled cells at 37°C for 30 min many macrophages lost their DR label but dendritic cells always retained some surface label. Some gold labelling DR and DP was found in characteristic channels between the veils and became internalized in membrane-bound structures. A small proportion of the RFDI label internalized in areas resembling coated pits. Less DQ label internalized and appeared on vesicles inside vacuoles. Material bound to different class II molecules may thus be internalized or processed differently by dendritic cells. The presence in inflammatory lesions of large activated dendritic cells with high expression of class II antigens suggests that these cells could be presenting antigen to lymphocytes within the joints.

Keywords antigen presenting cells in inflammation inflammatory arthritis juvenile chronic arthritis histocompatibility antigens in inflammation

INTRODUCTION

Specialized antigen presenting cells, particularly dendritic cells, are required in the initiation of primary immune responses (for reviews see Steinman & Nussenzweig, 1980; Knight, 1984; Steinman *et al.*, 1986; Austyn, 1987) and are involved in the maintenance of inflammatory reactions (Knight *et al.*, 1983; 1988a). The increase of these dendritic cells within the synovial membrane and fluids of patients with inflammatory arthritis (see Knight, 1988b) suggests that they may be involved in the maintenance of immunity, perhaps by the continuous presentation of antigens to lymphocytes within the joints. The inflammatory lesions in rheumatoid arthritis can resemble those of delayed hypersensitivity reaction. In the development of delayed hypersensitivity to contact sensitizer in mice the antigen is known to be presented to the immune system by dendritic cells (Macatonia *et al.*, 1987). In this latter system, dendritic cells acquire high levels of antigen, have a more activated appearance than resting dendritic cells with a larger Golgi region and more lysosomes and microfilaments, and can stimulate T cell responses. We have therefore investigated the appearance of the dendritic cells in synovial fluids, using electron microscopy to see whether they resemble these 'activated' dendritic cells. In addition, since class II expression is a prerequisite for antigen presenting function, we have studied the expression of D locus antigens on dendritic cells from the synovial fluids and compared it with that previously observed on dendritic cells from normal peripheral blood (Knight *et al.*, 1987; Brooks & Moore, 1988). Cells expressing high levels of HLA-DQ antigen in addition to HLA-DR are particularly potent antigen-presenting cells (Conte *et al.*, 1982; Gonwa *et al.*, 1983; Falk *et al.*, 1983; Nunez *et al.*, 1984, 1987) and HLA-DQ may have an important immunoregulatory influence on immune responses (Matsushita *et al.*, 1987) so the expression of these antigens both on DC and on macrophages from the joints was examined. Patients with juvenile chronic arthritis frequently had high numbers of dendritic cells in their synovial fluid (Tyndall, Knight & Edwards, 1983; Harding & Knight, 1986) and most of our studies used cells from these patients. More limited studies of dendritic cells from adult patients with rheumatoid arthritis

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showed that these cells had similar properties. Dendritic cells from synovial fluid and from peripheral blood expressed high levels of all the D locus antigens, whereas macrophages expressed only high levels of DR. However, dendritic cells from synovial fluids were larger and had an extensive Golgi region and many lysosomes, suggesting that they were activated. This supports the idea that they may be presenting antigen within the joints.

MATERIALS AND METHODS

Patients

Cells were from the joint fluids of patients with juvenile chronic arthritis with cellular joint fluids (Harding & Knight, 1986). Only three samples had sufficient cells to label with all four HLA-D locus antibodies, and the electron microscope pictures and comparisons of labelling were made with these. However, additional samples from three other patients with juvenile chronic arthritis and from four adults with rheumatoid arthritis were labelled with antibodies to DR or to RFDI only. These showed similar labelling patterns, suggesting that the findings are not confined to patients with very high numbers of cells in the joint fluids. Patients were receiving non-steroidal anti-inflammatory drugs.

Cell suspension

Mononuclear cells from synovial fluid were isolated on Ficoll gradients, cultured overnight on plastic Petri dishes (Nunc, Roskilde, Denmark) in medium (RPMI 1640; Dutch modification, Flow Laboratories, with 7·5% fetal calf serum, FCS) and non-adherent cells were centrifuged at 600 ***g*** for 10 min over a gradient of 14·25% (w/v) metrizamide (analytical grade, Nyegaard, Oslo, Norway) in medium. The low-density non-adherent mononuclear cells from the interface contained around 30% dendritic cells and 70% macrophages (Harding & Knight, 1986; Knight *et al*, 1987). In the electron microscope dendritic cells were identified by their pale cytoplasm, frequent projecting veils with few organelles, lobed nuclei and low content of phagolysosomes. This was distinct from the macrophages with their darker cytoplasm and prominent phagolysosomes. Some large mononuclear cells (<25%) were not clearly identifiable. In many samples, there were very few lymphocytes but occasionally significant numbers of contaminating lymphoblasts (<20%) were present as identified in some samples from their sensitivity to anti-T cell antibody and complement or in electron microscopy by their typical morphology with large nuclei with characteristic chromatin patterns and prominent nucleoli. Dendritic cells enriched from the peripheral blood of patients were similar to those already described in normal peripheral blood (Knight *et al.*, 1986) and therefore are not described further in this report.

Labelling cells

Low-density cells in phosphate-buffered saline (PBS) containing 0·1% azide were divided into 50-μl aliquots containing approximately 200 000 cells. Antibodies were an anti-DR antibody and the anti-DQ antibody Leu 10 from Beckton Dickinson (Mountain View, CA), the anti-DP antibody P 11.1 kindly provided by Dr J. Bodmer and the anti-class II antibody RFDI (which labels a two-chain structure typical of a D-locus product, has a distribution similar to DQ and is reported to be preferentially expressed on dendritic cells (Poulter *et al.*, 1982; 1986) which was a gift from Dr L. Poulter. For labelling (at 4°C) antibody (5 μl) was added for 30 min, the cells were washed twice and then incubated for 30 min with colloidal gold particles of 20 nm conjugated with staphylococcal protein A (Roth, 1982). These bound to all antibodies except RFDI which is an IgM antibody, and was labelled using 5-nm gold particles conjugated with anti-mouse IgM antibody or with a second layer rabbit anti-mouse serum and 20 nm gold with protein A. The samples were incubated at 4°C or at 37°C for 30 min, washed twice, fixed in 3% glutaraldehyde in 0·1 M cacodylate buffer, post-fixed in osmium tetroxide, dehydrated and embedded in Spurr resin. Cells were observed in a Jeol (Tokyo, Japan) 12.00 EX electron microscope. Control samples that were incubated with gold/protein A complex in the absence of antibody with an irrelevant monoclonal antibody, or with the IgM antibody RFDI showed no gold labelling in samples kept at 4°C. In samples held at 37°C in the presence of gold, around 10% of the cells showed very low labelling (3–12 gold particles) which was on vesicles in vacuoles.

RESULTS

General appearance of dendritic cells from synovial fluids

The appearance of the dendritic cells from synovial fluids is illustrated in Fig. 1a, where the large Golgi region, many lysosomes and microfilaments can be seen. Most of the dendritic cells in the samples from synovial fluids were these large cells of activated appearance. Although formal measurements of cell size were not attempted on these irregularly shaped cells, it was evident that dendritic cells from synovial fluids were larger than most of those from peripheral blood (Knight *et al.*, 1987), with longer veils, more extensive Golgi regions and more lysosomes. Large cytoplasmic granules (possibly lipid) were seen in some dendritic cells from synovial fluids (Fig. 1a). Similar granules were seen in dendritic cells from peripheral blood only after several days in culture (Patterson & Knight, 1987).

Labelling with RFDI

Most dendritic cells labelled with this antibody (Fig. 1a), which was usually seen in small patches on the surface membrane. Some patches of gold were at points of contact between cells or at points of contact between veils on a single cell (Fig. 1b). None of the cells with clear macrophage morphology with obvious phagocytic vacuoles showed significant labelling with this antibody. On incubating the labelled samples at 37°C, much of the gold remained on the surface of the cells but some was internalized through areas resembling coated pits (Fig. 1c).

Labelling of DR

Labelling with anti-DR antibody was seen on all dendritic cells and on all phagocytic cells. This was also often present in areas of cell contact (not shown). On warming the labelled dendritic cells, channels containing label formed, apparently between the

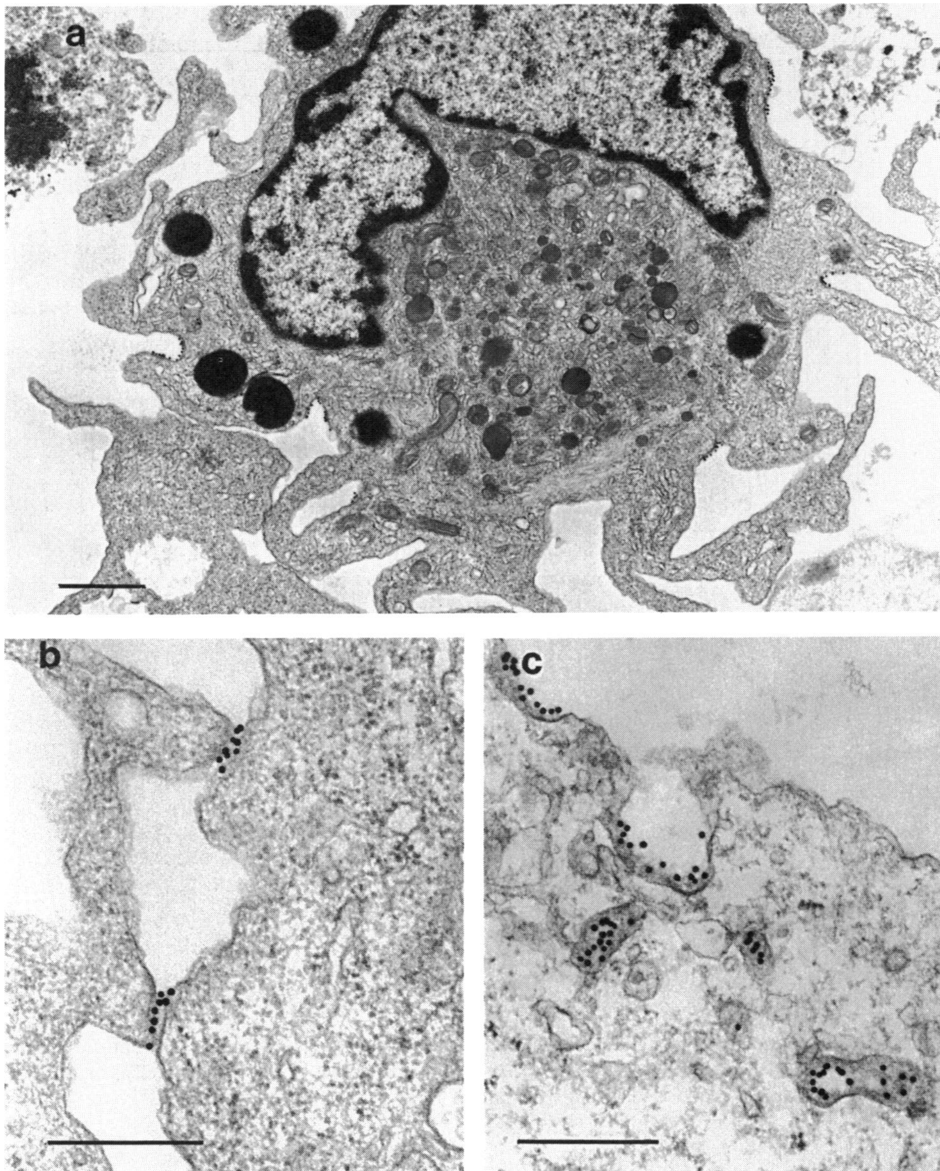

Fig. 1. Dendritic cells from the synovial fluid of patients with juvenile chronic arthritis were labelled with the IgM antibody RFDI, rabbit anti-mouse IgM and 20 nm gold particles coated with protein A. (a) Labelled at 0°C. × 11 000. Bar = 1 μm; (b) Labelled at 0°C. × 42 000. Bar = 0·5 μm; and (c) Incubated at 37°C for 30 min. × 37 500. Bar = 0·5 μm.

veils of the cells (Fig 2a and b). Some of the gold appeared to be completely internalized in membrane-bound channels (Fig. 2c). This channelled DR was seen in 75% of the dendritic cells. However, some DR label always remained on the surface of the dendritic cells. Some DR labelling macrophages also showed channelling of the label but most of the macrophages completely lost their label on incubation at 37°C.

Labelling of DQ

Diffuse labelling of DQ was seen on > 70% DC (Fig. 3a) but virtually no labelling of macrophages was observed. The label was not concentrated at points of cell contact like RFDI and DR. On incubation, the label mainly remained on the surface of dendritic cells, but occasional internalization on vacuoles in vesicles was seen (Fig. 3a).

Labelling of DP

DP was again detected on most dendritic cells but was virtually absent from macrophages. There was concentration of the antigen at points of cell contact or at points of contact between veils on the surface of the same cell. On warming, much of the label again remained on the surface of the cells but some showed a little channelling and internalization into small, membrane-bound vesicles within the cytoplasm of the cells. The labelling and channelling with DP often resembled that seen with DR. (Fig. 3b, c).

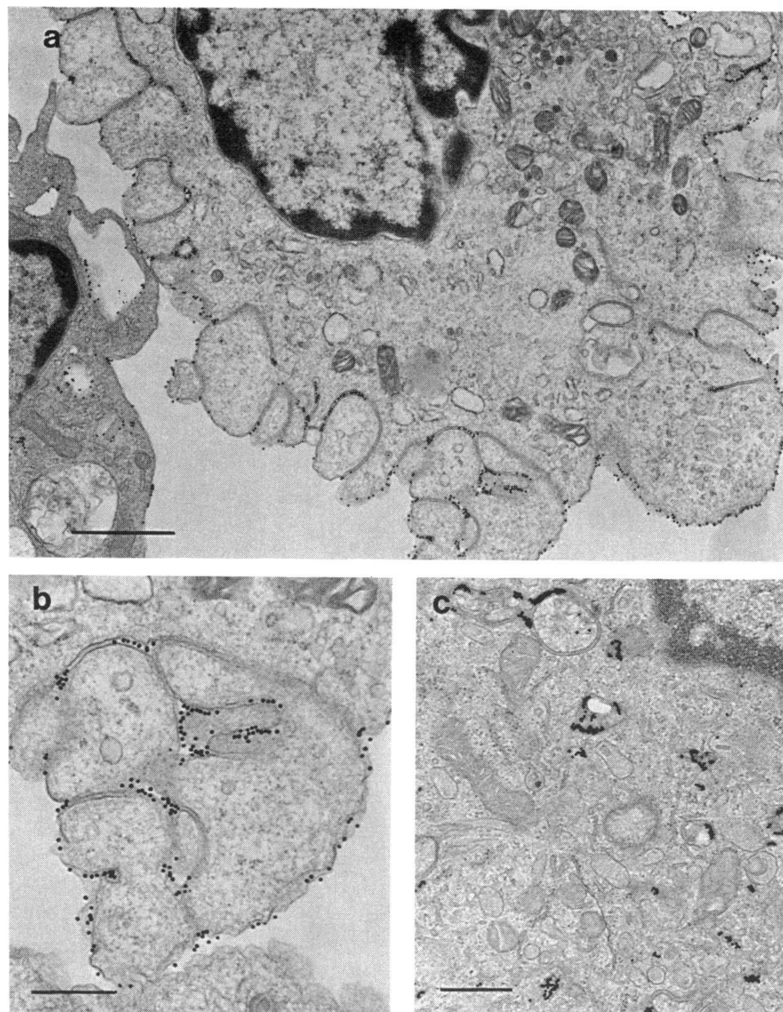

Fig. 2. Dendritic cells from the synovial fluid of patients with juvenile chronic arthritis were labelled with anti-DR antibody plus 20 nm gold particles coated with protein A. (a) Incubated at 37°C for 30 min. × 14000. Bar = 1 μm; (b) same cell as in (a), × 23000. Bar = 0·5 μm; and (c) incubated at 37°C for 30 min. × 19000, Bar = 0·5 μm.

DISCUSSION

The dendritic cells in the joint fluids from patients with inflammatory arthritis were larger than those in peripheral blood in healthy subjects or patients and expressed high levels of class II antigens. The large size of these cells and their well-developed Golgi regions, abundant lysosomes and microfilaments were features previously seen in mouse lymph node dendritic cells that had acquired antigen which they were able to present to T lymphocytes during the initiation of delayed hypersensitivity to a contact sensitizer (Macatonia *et al.*, 1987). In the synovial fluid of patients with inflammatory arthritis, there was also an increased proportion of dendritic cells in the mononuclear cell population, compared with that found in the synovial fluids in osteoarthritis (Harding & Knight, 1986) or in the peripheral blood (Zvaifler *et al.*, 1985; Harding & Knight, 1986). Since the only known function of these cells is to present antigen, this adds weight to the idea that these cells are presenting antigens to the T cells within the joints. The initiation of immune responses within the lymph nodes follows the influx of antigen-bearing dendritic cells and it is possible that a similar sequence of events is responsible for the development of the inflammatory lesions within the joints.

The presence on the cells of high levels of all the class II products studied is a feature that distinguished the dendritic cells from the macrophages in the low density cell population. The macrophages only expressed high levels of DR. As seen previously in peripheral blood, the dendritic cells and not the macrophages have high levels of DP and DQ antigens (Knight *et al.*, 1987; Brooks & Moore, 1988). Some studies indicate that cells expressing high DQ in addition to DR are particularly potent antigen-presenting cells (Conte *et al.*, 1982; Gonwa *et al.*, 1983; Nunez *et al.*, 1984). In our studies, the macrophages also showed little labelling with RFDI. This antibody has also been shown to be expressed on dendritic cells and not on macrophages in low-density cells from normal peripheral blood (Knight *et al.*, 1987) and in tissue sections (Poulter *et al.*, 1982; 1986). However, there is also a report that dendritic cells within the joints lack RFDI (Waalen *et al.*, 1987) and another that there are cells expressing both macrophage markers and RFDI

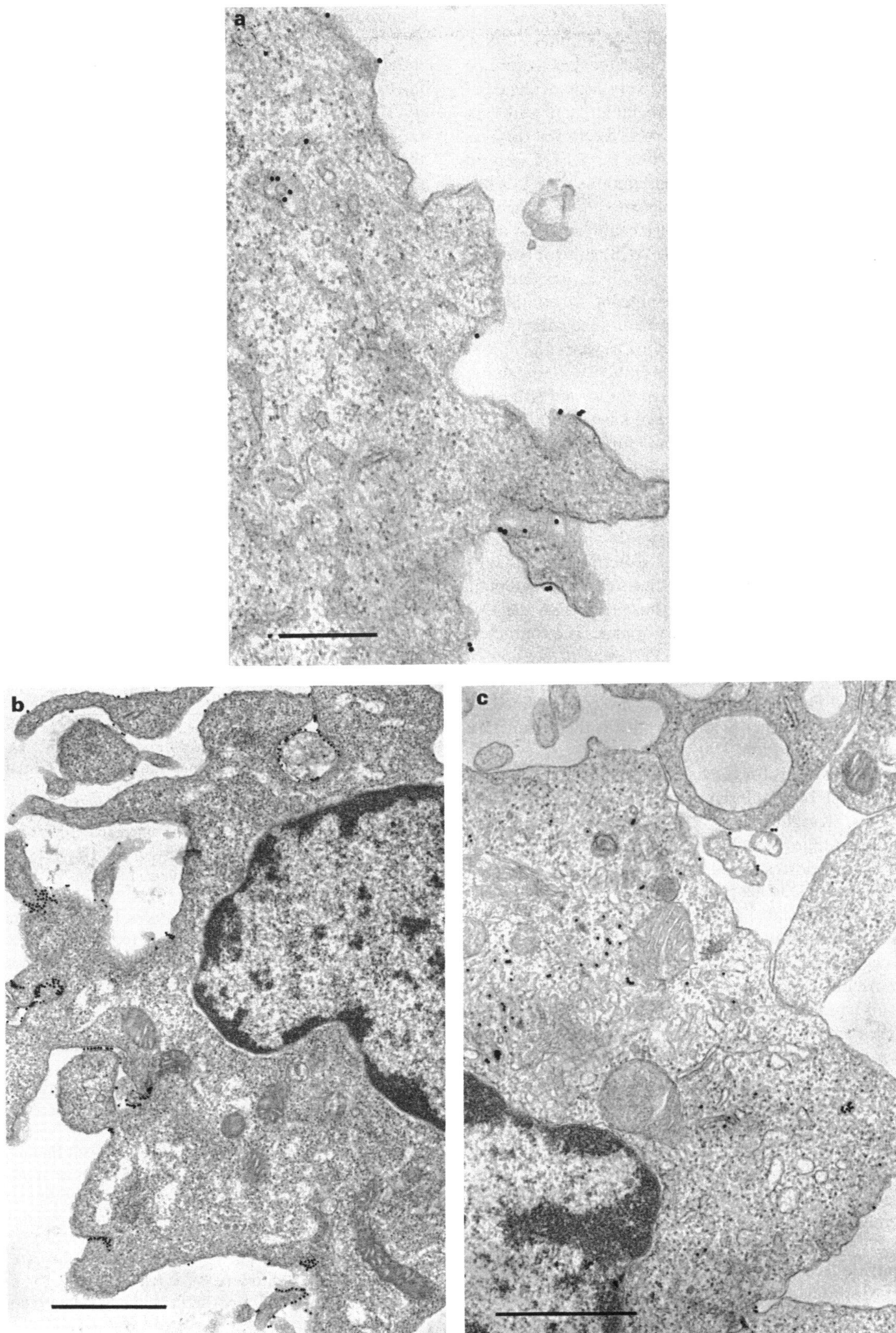

Fig. 3. Dendritic cells from patients with juvenile chronic arthritis labelled with antibodies to DQ or DP plus 20 nm gold particles coated in protein A. (a) DQ-labelled cell incubated at 37°C for 30 min. ×36000. Bar = 0·5 μm; (b) DP-labelled cell incubated at 37°C for 30 min. × 18750. Bar = 1 μm; and (c) DP-labelled cell incubated at 37°C for 30 min. ×24000. Bar = 1 μm.

present particularly in the synovial linings of patients with inflammatory arthritis (Salisbury, Duke & Poulter, 1987). However, using a double-labelling technique for RFDI and Leu M3 (a macrophage antibody) we found very little evidence of any cells in synovial fluids with double labelling. There were very occasional spots of RDFI on Leu M3-labelled cells but this was only a very small fraction of the labelling for RFDI seen on dendritic cells. However, when these populations were labelled with D locus antibodies using a fluorescein-conjugated second antibody, the dendritic cells showed very heavy labelling using the fluorescence-activated cell sorter (FACS) but monocytes/macrophages (identified with Leu M3 directly conjugated with phycoerythrin) did give low but significant labelling (not shown). RFDI has been shown to label an antigen with a tissue distribution similar to that of HLA-DQ (Drexler *et al.*, 1988) but is probably coded at separate locus (Janossy, Campana & Bollum, 1986). However, RFDI blocked the mixed leukocyte reaction (Poulter *et al.*, 1986) which suggests that it may be important in presenting antigen to T cells. Our own studies show a distinct distribution of RFDI in patches which are generally smaller and more discrete than those of other D locus products. The differences in labelling and internalization of RFDI and DR were not due to the fact that RFDI was an IgM antibody and the anti-DR was IgG since in separate experiments an IgM antibody to DR was shown to give the same characteristic labelling and channelling patterns as those seen with the IgG antibody. The paucity of RFDI on macrophages is a characteristic shared with HLA-DP and -DQ. Preferential location of RFDI found at points of cell/cell interaction is also a feature seen with DR and DP. The nature of the molecule defined by RFDI, its relation with other D locus products and its function still require further definition.

The labelling patterns for the dendritic cells of the synovial fluid thus resembled very closely those already described for peripheral cells (Knight *et al.*, 1987), although the larger dendritic cells from the joints had generally more label per cell. The current observations showed that, within the joints dendritic cells express more D locus products than macrophages. The internalization of some label in warmed dendritic cells suggests that they may internalize and, perhaps, 'process' antigens bound to different class II molecules. Other evidence for internalization of class II molecules has also been obtained using gold/antibody conjugates and Langerhans cells (Hanau *et al.*, 1987) or by measuring the increase in the cycloheximide-persistent, internalized I-A antigen labelled with monoclonal antibodies in populations of mouse peritoneal exudate cells (Harding & Unanune, 1989). However, we show that all dendritic cells also retained some class II label on the surface of the cells while many macrophages completely lost their DR on warming. Compared with macrophages the dendritic cells therefore appear to have a more persistent expression of all class II molecules, which could contribute to the maintenance of antigen presentation within the joints.

The dendritic cells from the joints of some patients have also been shown to stimulate autologous lymphocytes in an autologous mixed leucocyte reaction which is another reactivity dependent on class II molecules (Harding & Knight, 1986). There is evidence that this may represent mainly the presentation of antigens acquired by dendritic cells to antigen-sensitive lymphocytes (Knight, 1988a) and so this provides further support for the idea that dendritic cells accumulating within the synovium and synovial fluids in inflammatory arthritis may be able to stimulate T cell proliferation. Studies of experimentally induced autoimmunity show the dendritic cells play a continuing role in presenting autoantigens to produce disease (Knight *et al.*, 1983; 1988). Our current work showing the activated state of the dendritic cells their high levels of class II molecules supports the theory that these specialized antigen-presenting cells in the joints are also initiating and promoting the inflammatory reactions.

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REFERENCES

Austyn, J.M. (1987) Lymphoid dendritic cells. *Immunology*, **62**, 161.

Brooks, C. & Moore, M. (1988) Differential MHC class II expression on human peripheral blood monocytes and dendritic cells. *Immunology*, **63**, 303.

Conte, G., Moretta, A., Cosulick, M.E., Ramali, D. & Bargellesi, A. (1982) A monoclonal anti-DCI antibody selectively inhibits the generation of effective T cells mediating specific cytolytic activity. *J. exp. Med.* **156**, 1539.

Drexler, H.G., Gignac, S.M., Brenner, M.K., Coustan-Smith, E., Janossy, G. & Hoffbrand, A.V. (1988) Differential expression of MHC class II antigens in chronic B-cell disorders. *Clin. exp. Immunol.* **71**, 217.

Falk, J.A., Cannon, M., Nakuoda, A., Lewis, W., Letarte, M. & Falk, R.E. (1983) Monoclonal antibody to MT inhibits antibody production. *Hum. Immunol.* **8**, 291.

Gonwa, T.A., Picker, L.J., Raff, H.V., Goyert, S.M., Silver, J. & Stobo, J.D. (1983) Antigen-presenting capabilities of human monocytes correlates with their expression of HLA-DS, a determinant distinct from HLA-DR. *J. Immunol.* **130**, 706.

Hanau, D., Fabre, M, Schmitt, D.A., Garaud, J.C., Pauly, G., Tongio, M.A., Mayer, S. & Cazenave, J.P. (1987) Human epidermal 'Langerhans' cells co-internalize by receptor-mediated endocytosis 'non-classical' major histocompatibility complex class I molecules (T6-antigens) and class II molecules (HLA-DR antigens) *Proc. natl. Acad. Sci. USA*, **84**, 2901.

Harding, C.V. & Unanue, E.R. (1989) Antigen processing and intracellular Ia. Possible rates of endocytosis and protein synthesis in Ia function *J. Immunol.* **142**, 12.

Harding, S. & Knight, S.C. (1986) The distribution of dendritic cells in the synovial fluids of patients with arthritis. *Clin. exp. Immunol.* **63**, 594.

Janossy, G., Campana, D. & Bollum, F. (1986) Immunofluorescence studies in leukaemia diagnosis. In *Monoclonal Antibodies* (ed. by P.C.L. Beverley) p. 97 Churchill-Livingstone, Edinburgh.

Knight, S.C. (1984) Veiled cells—'dendritic cells' of the afferent lymph. *immunobiology*, **168**, 348.

Knight, S.C. (1988a) The autologous mixed leucocyte reaction and dendritic cells. (Editorial). *Br. J. Rheumatol.* **26**, 85.

Knight, S.C., (1988b) Dendritic cells in inflammatory joint disease. In *Immunopathogenetic Mechanisms of Arthritis* (ed. by T.A. Goodäcre and W.C. Dick) p. 69, MTP Press, Lancaster.

Knight, S.C., Farrant, J., Bryant, A., Edwards, A.J., Burman, S., Lever, A., Clarke, J. & Webster, A.D.B. (1986) Non-adherent, low-density cells from human peripheral blood contain dendritic cells and monocytes, both with veiled morphology. *Immunology*, **57**, 595.

KNIGHT, S.C., FARRANT, J. CHAN, J. BRYANT, A., BEDFORD, P.A. & BATEMAN, C. (1988) Induction of autoimmunity with dendritic cells: studies on thyroiditis in mice. *Immun. Immunopathol.* **48,** 277.

KNIGHT, S.C., FRYER, P., GRIFFITHS, S. & HARDING, B. (1987) Class II histocompatibility antigens of human dendritic cells *Immunology,* **61,** 21.

KNIGHT, S.C., MERTIN, J., STACKPOOLE, A. & CLARKE, J. (1983) Induction of immune responses *in vivo* with small numbers of veiled cells. *Proc. natl. Acad. Sci. USA,* **38,** 6032.

LECHLER, R.I. & BATCHELOR, J.R. (1982) Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. *J. exp. Med.* **115,** 31.

MACATONIA, S.E., KNIGHT, S.C., EDWARDS, A.J., GRIFFITHS, S. & FRYER, P. (1987) Localisation of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate *J. exp. Med.* **166,** 1654.

MATSUSHITA, S., MUTO, M. SUEMURA, M., SAITO, Y. & SASUZUKI, T. (1987) HLA-linked non-responsiveness to Cryptomeria japonica pollen antigen I non-responsiveness is mediated by antigen-specific cells. *J. Immunol.* **138,** 139.

NUNEZ, G., BALL, E.J., MYERS, L.K. & STASNY, P. (1984) Expression of HLA-DQ molecules on human monocytes: populations with distinct accessory cell function. In *Histocompatibility Testing* (ed by E.D. Albert, M. Baur & W.R. Mayr) p. 636. Springer-Verlag, Berlin.

NUNEZ G., BALL, E.J. & STATSNY, P. (1987) Antigen presentation by adherent cells from human peripheral blood. Correlation between T cell activation and expression of HLA-DQ and DR antigens. *Hum. Immunol.* **19,** 29.

PATTERSON, S. & KNIGHT, S.C. (1987) Susceptibility of human peripheral blood dendritic cells to infection by human immunodeficiency virus. *J. gen. Virol.* **68,** 1177.

POULTER, L.W., CAMPBELL, D.A., MUNRO, C. & JANOSSY, G. (1986) Discrimination of human macrophages and dendritic cells by means of monoclonal antibodies. *Scand. J. Immunol.* **24,** 351.

POULTER, L.W., DUKE, O., HOBBS, S., JANOSSY, G. & PANAYI, G.S. (1982) Histochemical discrimination of HLA-DR positive cell populations in normal and arthritic synovial linings. *Clin. exp. Immunol.* **48,** 381.

ROTH, J. (1982) A protein A-gold technique—a qualitative and quantitative approach for antigen localisation in thin sections. In *Techniques in Immunocytochemistry* vol. 1 (ed. by G.R. Bullock & P. Petruez) p. 107. Academic Press, London.

SALISBURY, A.K., DUKE, O. & POULTER, L.W. (1987) Macrophage-like cells of the Pannus area in rheumatoid arthritic joints. *Scand. J. Rheumatol.* **16,** 263.

STEINMAN, R.M. & NUSSENZWEIG, M. (1980) Dendritic cells—features and functions. *Immunol. Rev.* **53,** 127.

STEINMAN, R.M., INABA, K., SCHULER, G. & WITMER, M. (1986) Stimulation of Immune responses—contributions of dendritic cells. In *Host Resistance mechanism to Infectious Agents, Tumors and Allografts.* (ed. by R. M. Steinman & R. J. North) p. 71. Rockefeller University Press, NY.

TYNDALL, A., KNIGHT, S.C. & EDWARDS, A. (1983) Veiled (dendritic) cells in synovial fluid. *Lancet,* **i,** 472.

WAALEN, K., FORRE, O.P., PAHLE, J., NATVIG, J.B. & BURMESTER, C.R. (1987) Characteristics of human synovial and normal blood dendritic cells. Retention of class II MHC antigens and accessory function after short-term culture. *Scand. J. Immunol.* **26,** 525.

ZVAIFLER, N.J., STEINMAN, R.M., KAPLAN, G. LAU, L.L. & RIVELIS, M. (1985) Identification of immunostimulatory dendritic cells in the synovial effusions of patients with rheumatoid arthritis. *J. Clin. Invest.* **76,** 789.