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The distribution of lymphoid and macrophage like cell subsets of sarcoid and Kveim granulomata: possible mechanism of negative PPD reaction in sarcoidosis

B. B. MISHRA,*† L. W. POULTER,† G. JANOSSY† & D. GERAINT JAMES*
**Department of Medicine, Royal Northern Hospital and †Department of Immunology, Royal Free Hospital School of Medicine, London, UK.*

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SUMMARY

Immunohistological observations of lymphoid and non-lymphoid cell subsets in biopsies of sarcoid skin granulomas have been compared with positive Kveim tests and the sites of PPD injection in sarcoid patients. Monoclonal antibodies have been used in indirect immunofluorescence often in combination with histochemical methods for the detailed characterization of the cells involved. The antibodies used included two new reagents, RFD-1 and RFD-2, which react with interdigitating cells and acid phosphatase positive macrophages, respectively. Sarcoid granulomas had a distinctive pattern of organization though there was a heterogeneity of macrophage like and T lymphoid cells. In the centre, predominantly HLA-DR$^+$, acid phosphatase positive macrophages (RFD-2$^+$) were seen and the lymphoid cells were almost exclusively T4$^+$. At the periphery of the granulomas the HLA-DR$^+$ dendritic cells were ACP negative and RFD-1$^+$. Here T8$^+$ cells were admixed with the T4$^+$ population. The Kveim granuloma had fewer RFD-2$^+$ macrophages and therefore the RFD-1$^+$ cells were more evenly distributed, but the other cells showed a similar distribution to the established lesions. The PPD injection sites contained fewer T cells than the normal control infiltrates in PPD positive healthy individuals. The T4$^+$/T8$^+$ ratios were about 3:2. The most likely explanation for the PPD anergy in sarcoidosis is the sluggish traffic of T4$^+$ cells which could be due to the sequestration of T4$^+$ cells in sites of ongoing inflammation.

Keywords sarcoidosis immunopathology granulomata T cells macrophages

INTRODUCTION

The progression of sarcoidosis is associated with disturbances of the body's immunoregulating mechanisms (James, Neville & Walker, 1975). A loss of skin test reactivity to PPD and other immunogens is frequently observed in sarcoid patients (James, 1966) along with impairment of the *in vitro* proliferative responses of blood mononuclear cells to antigens and mitogens (Sharma, James & Fox, 1971). Cutaneous reactivity to a sarcoid spleen suspension (Kveim test) is present in three quarters of patients (James, Thomson & Willcox, 1956).

As the only histopathological feature of sarcoidosis is the granuloma, it is reasonable to suggest that it is here that the pathological manifestations of the immunoregulatory disorders will be reflected.

Correspondence: Dr L. W. Poulter, Department of Immunology, Royal Free Hospital School of Medicine, Pond Street, London NW3 2QG, UK.

Light and electron microscopy have been used to examine cell populations within the granulomas and have established the presence of epithelioid cells, macrophages, and lymphocytes (Thomson, 1958). More recently antibodies to functionally distinct lymphocyte subsets have been developed (Rheinerz & Schlossman, 1980) and used in histology (Janossy *et al.*, 1980). These methods have also been applied in combination with histochemical techniques and are useful in identifying macrophage subpopulations within normal and pathological tissues (Poulter *et al.*, 1982). The ability to carry out such analyses in tissue sections allows the spatial interrelationships of different cell types to be examined.

The present study applies such techniques to punch biopsies of cutaneous sarcoid lesions and sites of Kveim and PPD skin test reactions. Three questions were asked: (1) what is the relationship of the $T4^+$ and $T8^+$ T cell populations to the different macrophage and epithelioid elements in the lesions? (2) Does the Kveim test induce granulomas similar to the sarcoid granuloma by these newly established criteria? Finally, (3) what is the cellular basis of a negative PPD reaction in patients with active sarcoidosis?

MATERIALS AND METHODS

Clinical material. In all, 16 patients were studied (Table 1). Biopsies of sarcoid skin lesions were taken from seven patients with clinical radiographical and histological evidence of sarcoidosis. None of these had received immunosuppressive therapy for at least 3 months prior to the biopsies.

Five sarcoid patients received 10 tuberculin units of PPD intradermally and full thickness skin biopsies were taken from the injected site after 48 h. The PPD reactions were regarded positive when the induration was more than 5 mm in diameter 48 h after the injection of the antigen. The skin reactions were compared to those seen after the injection of the same amount of PPD intradermally into PPD positive individuals (Poulter *et al.*, 1982).

Kveim reactions were biopsied in six patients with sarcoidosis 4 weeks after an intradermal injection of Kveim reagent. Normal skin was obtained from healthy volunteers. The clinical details are listed on Table 1.

Preparation of tissue sections. All blocks were covered in OCT compound and snap frozen in isopentane pre-cooled in liquid nitrogen. Six micrometre sections were cut using a cryostat at $-25^{\circ}C$, air dried at room temperature for 2 h, fixed for 5 min in chloroform/acetone (1:1), freeze dried and stored at $-20^{\circ}C$.

To confirm the pathology and demonstrate the morphology, some sections from each specimen were stained with haematoxylin and eosin (H & E).

Immunofluorescence and histochemistry. Indirect immunofluorescence and combination staining with immunofluorescence and histochemistry was carried out as previously described (Poulter *et al.*, 1983). The antibodies used are listed in Table 2.

Quantitation. Quantitation of Langerhans cells was performed by labelling sections with a combination of monoclonal antibody NAl/34 and a heterologous antiserum to cytokeratin kindly supplied by Dr L. Trejdosiewicz (Table 2). The number of Langerhans cells per 100 basal layer cells was determined by counting randomly selected fields of at least two sections from all biopsies of appropriate groups (Poulter *et al.*, 1982).

$HLA\text{-}DR^+$, ACP^+ cells were counted in at least three sections from each biopsy using combined histochemistry and immunofluorescence. The means and standard deviations of the number of cells with this phenotype in sarcoid granuloma, Kveim granuloma and in biopsies of negative PPD sites were calculated. The same process was repeated to obtain the mean numbers and standard deviations of $HLA\text{-}DR^+$, ACP^- cells in each of the three categories of biopsies studied. The number of $RFD\text{-}1^+$ cells in consecutive sections was added to the total population of $HLA\text{-}DR^+$, ACP^- cells to obtain the mean numbers and standard deviations of cells with the phenotype $HLA\text{-}DR^+$, ACP^-, $RFD\text{-}1^+$.

Controls. Sections of tonsils were used as positive controls for the first layer antibodies. Sections of the skin biopsies incubated with only the second layer antibodies served as negative controls.

Table 1. Clinical features of sarcoid patients included in the study

Name	Sex	Age at onset	Chest X-ray stage	Skin lesions	Eye lesions	Histology	Others	SACE	Tissue used
VR	F	27	0	Plaques	Absent	Liver granuloma	Splenomegaly	92	Skin lesion
PD	F	27	I	Maculopapular eruption; Erythema nodosum.	Absent	Skin granuloma		72	Skin lesion
AN	M	28	I	Papules; Erythema	Acute uveitis	Skin & Liver granulomas	Splenomegaly; Anaemia	244	Skin lesion
MH	M	50	II	Lupus pernio; Maculopapular eruption	Absent	Nasal mucosa and skin granulomas	Bone cyst	36	Skin lesion & PPD reaction site
SM	M	37	III	Subcutaneous nodules	Acute uveitis	Skin granuloma		101	Skin nodule
MM	M	52	II	Plaques	Acute uveitis	Skin & Kveim test granulomas	Bilateral parotitis	129	Skin lesion
AW	F	35	I	Maculopapular eruptions	Acute uveitis conjunc-tivitis	Liver & skin granulomas	Metropathia haemorrhagica	33	Skin lesion
AT	F	63	II	Erythema nodosum	Absent	Lymph node granuloma	Splenomegaly; peripheral lymph-adenopathy	69	PPD reaction
EW	F	36	I	Absent	Absent	Kveim granuloma	Bone cysts; subarachnoid haemorrhage	33	PPD reaction
EA	F	35	I	Absent	Acute uveitis	Liver granulomas	Gall stones	79	PPD reaction
GS	M	28	II	Absent	Absent	Kveim test granuloma		101	Kveim & PPD test site
AR	F	30	0	Absent	Acute uveitis	Kveim test granuloma	Anaemia	58	Kveim test site
PH	M	33	I	Absent	Absent	Kveim test & Liver granulomas	Hepatomegaly jaundice	69	Kveim test site
CM	M	48	0	Conjunctival follicle	Absent	Conjunctival granuloma	Cervical lymph-adenopathy	92	Kveim test site
WF	F	40	0	Absent	Absent	Liver & Kveim test granuloma		43	Kveim test site
FJ	F	55	III	Absent	Absent	Kveim test granuloma		106	Kveim test site

SACE = serum angiotensin converting enzyme (normal = less than 55 nmols/ml/min).

Table 2. Reagents used in the study

First layer antibody (Mouse monoclonal)	References	Specificity	Second layer antibody	Other antibody reagents used in combination
Anti-HLA-DR (IgG or IgM class)*	Prepared in RFH Janossy *et al.*, (1979, 1981)	Class II MHC antigens	G† anti-M† IgM or IgG TRITC	UCHTl, RFT8 TO 15
UCHT1 (IgG)*	Callard *et al.* (1981)	T lymphocytes	G anti-M IgG FITC	anti-HLA-DR (IgM) RFT8 IgM
OKT 4 (IgG)*	Janossy *et al.* (1980)	T helper subset	G anti-M IgG FITC	anti-HLA-DR (IgM)
RFT 8 (IgM)*	Prepared in RFH Janossy *et al.* (1980)	T suppressor/ cytotoxic subset	G anti-M IgM TRITC	OKT4 (IgG), UCHT1 (IgG)
NA1/34 (IgG)*	McMichael *et al.* (1979)	Langerhans cells, cortical thymocytes	G anti-M TRITC/ FITC	Heterologous (rabbit) anti-cytokeratin antibody
RFD1 (IgG)*	Poulter *et al.* (1983) Raftery *et al.* (1983)	Interdigitating cells of lymph nodes, few B cells	G anti-M TRITC/ FITC	
RFD2 (IgG)*	Poulter *et al.* (1983)	Macrophages	G anti-M TRITC	anti-HLA-DR
TO15	Stein, Gerdes & Mason (1982)	B cells	G anti-M TRITC	anti-HLA-DR

* Monoclonal antibodies made in mouse.
† G = goat; M = mouse.

RESULTS

Histology

H & E staining of the sarcoid skin lesions showed one or more characteristic granulomas with epithelioid cells and giant cells interspersed with lymphocytes centrally while other mononuclear cells with the characteristics of macrophages formed a peripheral mantle again interspersed with other lymphocytes. Each section contained from two to six granulomas of varying sizes; the smallest being an area of 200 cells.

The cutaneous infiltrates associated with the Kveim reaction were similar in appearance to the sarcoid lesions although much smaller and less well organised. Only one or two mostly subepidermal granulomas were seen on any one section of the Kveim reaction.

Despite the absence of any superficial erythema or induration, histological stains revealed small perivascular infiltrates at the PPD skin test sites. These varied from a total of 10–20 cells to 120–250 cells per high power field. The cells appeared to be mostly lymphocytes interspersed with small numbers of macrophage like cells. No epithelioid or giant cells were seen.

Immunohistological analysis of lymphoid subsets

Insignificant numbers of B cells (TO 15^+) were seen in any of the biopsies taken. On some sections of the sarcoid lesions very small numbers ($< 1\%$) of plasma cells were detected using heterologous anti-human kappa and lambda reagents (Table 2). Only occasional plasma cells or were detected in the biopsies of Kveim and PPD test sites.

In the sarcoid granulomas $UCHT1^+$ T cells were scattered diffusely and constituted approximately 33% of the cells. The relative proportion of the $UCHT1^+$ cells (T cells) in the Kveim

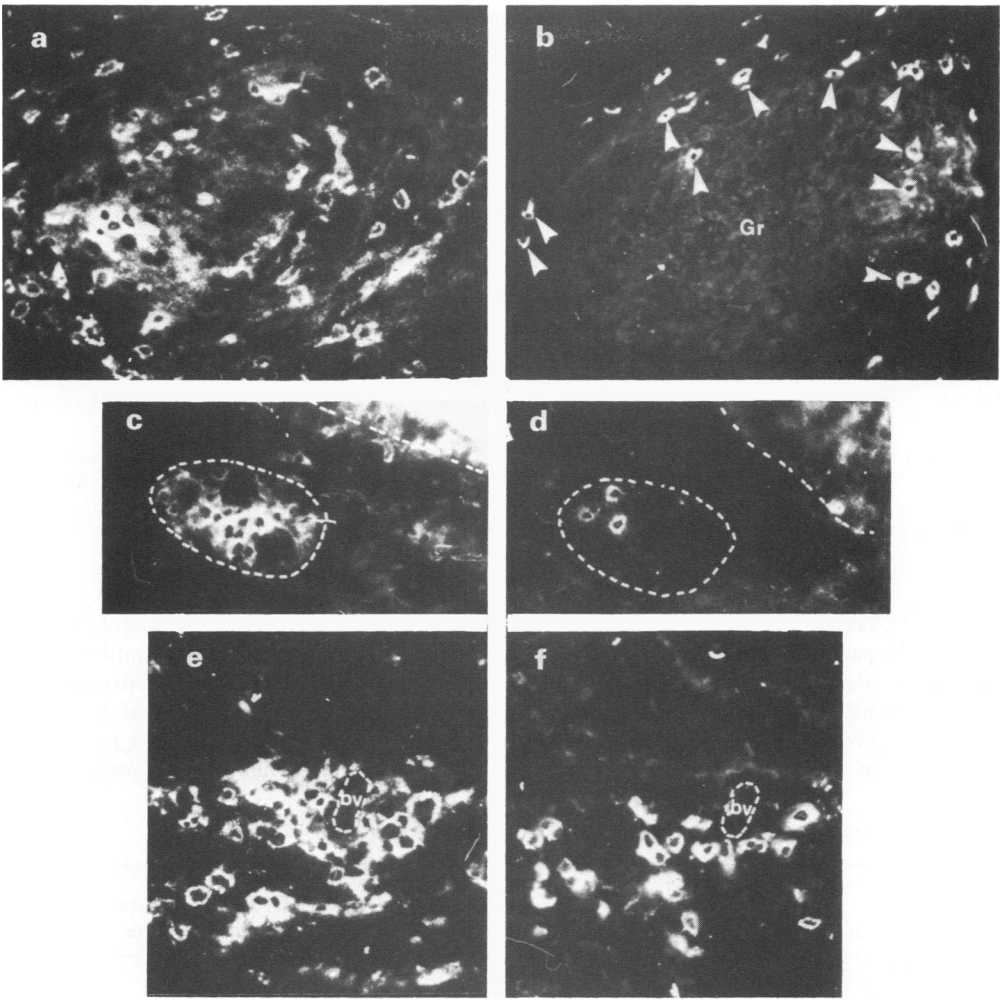

Fig. 1. Analysis of the distribution of $T4^+$ and $T8^+$ cells. (a) $T4^+$ lymphocytes distributed throughout the sarcoid granuloma. In the centre of the granuloma $T4^+$ cells are associated with non-lymphoid cells of epitheloid morphology. (b) $T8^+$ lymphocytes distributed in the periphery of the sarcoid granuloma and are found in the vicinity of HLA-DR^+, ACP^-, RFD-1^+ macrophage like cells (see also Figs 2 & 3). Gr = granuloma. (c) $T4^+$ lymphocytes in the granuloma of a positive Kveim test site are distributed throughout the Kveim granuloma resembling the distribution of $T4^+$ cells in the sarcoid granuloma (Fig. 1a). (d) $T8^+$ lymphocytes in the same granuloma as Fig. 1c. The $T8^+$ cells are predominantly found in the periphery of the Kveim lesion, as is seen in the sarcoid granuloma (Fig. 1b). (e) $T4^+$ lymphocytes at the site of a negative PPD reaction in a patient with sarcoidosis. (f) $T8^+$ lymphocytes at the same site as in Fig. 1e. The average $T4^+$:$T8^+$ ratio is 3:2 (Table 3).

granuloma was higher (40–50% of all cells present), than the number of T cells in a typical sarcoid lesion. Labelling with OKT4 and RFT8 monoclonal reagents revealed a spatial segregation within the granuloma. The $T4^+$ cells were found throughout the granuloma (Fig. 1a); thus in the centre of the granuloma they were situated in association with epithelioid cells.

The $T8^+$ cells showed an entirely different distribution, all of them appearing in the periphery of the granuloma (Fig. 1b). The ratio of $T4^+$ and $T8^+$ cells in the outer regions of granulomas was in the range of 4:1 to 6:1. This difference of $T8^+$ cell distribution between the middle and peripheral regions of granulomas was also seen in the biopsies of the Kveim test site (Figs 1c & d). Thus the overall proportion of $T4^+/T8^+$ cells in the whole Kveim lesion was around 4:1 (Table 3).

Table 3. Distribution of T and macrophage like cells in the skin of sarcoid patients

Cellular type		Sarcoid granuloma	Kveim granuloma	PPD negative reaction site
No. of patients		7	5	4
No. of sections counted	Phenotype	21	15	12
T lymphocytes	UCHT1$^+$ HLA-DR$^+$ TO 15$^-$	33% (±13%)	50% (±6%)*	40% (±11%)
Ratio of T lymphocytes				
Helper:suppressor type	T4:T8	5:1	4:1	3:2
Macrophages	HLA-DR$^+$ RFD-2$^+$ RFD-1$^-$	66% (±13%)	50% (±6%)	60% (±11%)

* Significantly different from sarcoid granuloma by students *t*-test ($P < 0{\cdot}025$). Differences in other counts were not statistically significant.

There was a considerable variation in numbers of infiltrating lymphocytes at the sites of the PPD skin tests in patients with sarcoidosis. UCHT1$^+$ T cells constituted 40% of all the infiltrating cells in the perivascular areas (Table 3). At the time of excision biopsy these sites showed a negative reaction to PPD. Comparison of the PPD-induced infiltration seen in sarcoid patients to that in normal individuals (Poulter *et al.*, 1982) reveals that a 48 h reaction in an active sarcoid patients is equivalent, in terms of cell numbers, to a 12 h reaction in a normal volunteer. In contrast to the

Table 4. Characteristics of macrophage like cells in the skin of patients with sarcoidosis

Cellular type		Sarcoid granuloma	Kveim granuloma	PPD negative reaction site
No. of patients		7	5	4
No. of sections counted	Phenotype	21	15	12
Activated* macrophages	HLA-DR +			
	RFD-2 +			
	RFD-1 −	54% (±27%)†	42% (±9%)	27 (±16%)
	ACP +			
	ATPase ±			
Interdigitating cell type	HLA-DR +			
	RFD-2 −			
	RFD-1 +	45% (±27%)	57% (±9%)	72% (±16%)
	ACP −			
	ATPase +			
Non-activated macrophages	HLA-DR −			
	RFD-2 +			
	ACP −	occasional	occasional	occasional
	ATPase ±			
	RFD-1 −			

* This group includes epithelioid cells and giant cells which have an identical phenotype but can be distinguished on morphological grounds.

† Proportion of total non-lymphoid cells in granuloma. Differences in the counts are not statistically significant.

Kveim and sarcoid lesions, the ratios of $T4^+$ and $T8^+$ cells at the site of PPD injection 48 h after PPD injection were 3:2 to 1:1 and showed no spatial segregation of the different subsets (Fig. 1e & f).

Finally, in the sarcoid granuloma lymphocytic infiltration was restricted to the dermis, whereas, in the biopsies of the Kveim and PPD reactions, some T cells were seen in the epidermis.

Non-lymphoid cells

The majority of the cells of the sarcoid granuloma were large irregular shaped macrophage like cells. When studied with immunological 'markers' the phenotype of these cells was HLA-DR^+, RFD-2^+,

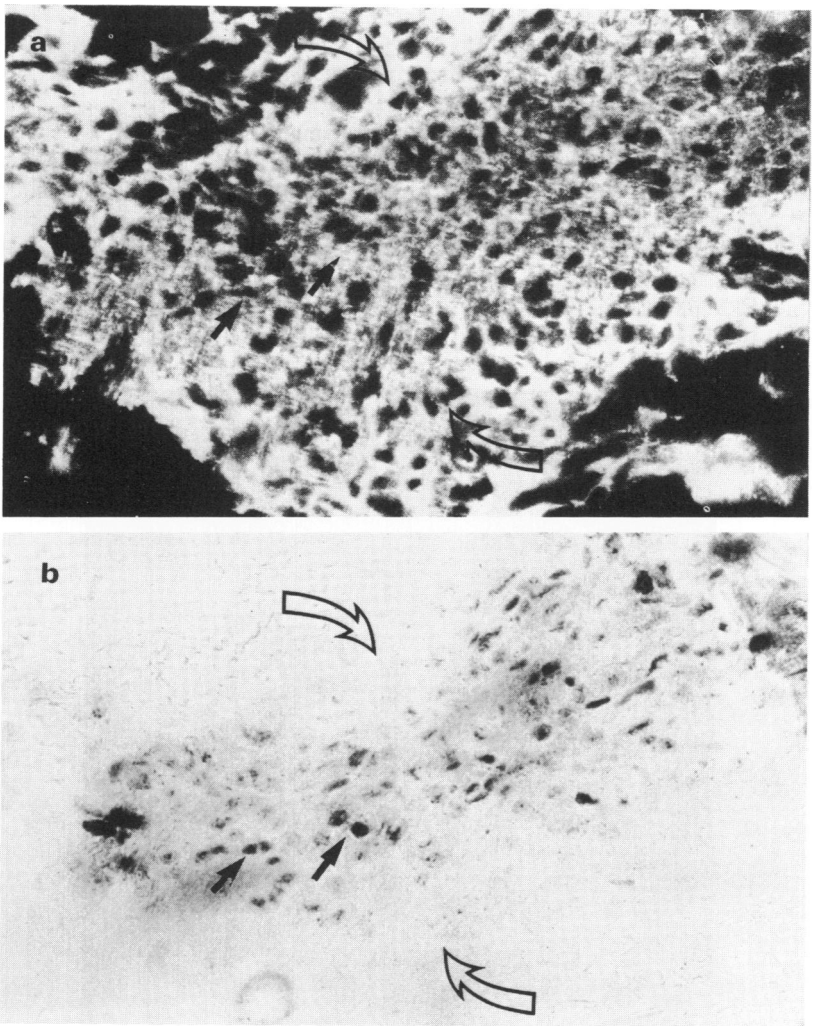

Fig. 2. Analysis of macrophage subsets in the sarcoid lesion by combined immunofluorescence for HLA-DR (a), and histochemistry for acid phosphatase (b). A majority of the cells in the centre of the sarcoid granuloma are HLA-DR^+ (small dark arrows). These HLA-DR^+ cells are macrophages as can be demonstrated by the presence of acid phosphatase activity (see Fig. 2b, small arrows) and by their reactivity with the macrophage specific monoclonal reagent RFD-2 (not shown here). The cell margins of the HLA-DR^+ cells are difficult to visualize because of the interdigitations between processes of neighbouring HLA-DR^+ macrophages. Individual cells are identified by the dark unstained nuclei. The peripheral areas show another type of HLA-DR^+ dendritic cells. These are acid phosphatase negative (large open arrows; Fig. 2b), but show reactivity with RFD-1 (see Fig. 3)

UCHT1$^-$ (Table 3). Approximately 66% of the cells of the granuloma had this phenotype. These cells could be further divided into three groups (Table 4). In the centre of the granulomas, epithelioid cells and multinucleate giant cells could be identified on morphological grounds. They expressed HLA-DR antigen (Fig. 2a), showed strong acid phosphatase (ACP) lysosomal enzyme activity (Fig. 2b) and were RFD-2 positive. The cell membranes of individual cells in the granuloma could not be distinguished by immunofluorescent staining of the membrane by anti-HLA-DR reagents. On the basis of these characteristics it is clear that the 'epithelioid' cells in the granulomas show macrophage like features such as reactivity with RFD-2 and strong ACP activity (type I cells; Table 4). The second group of non-lymphoid cells also expressed HLA-DR antigen but were ACP$^-$ ATPase$^+$. These cells were more frequent in the outer zones of the sarcoid granuloma in close association with T4$^+$ and T8$^+$ lymphocytes. The monoclonal reagent RFD-1 (Table 2) labelled non-lymphoid cells in a distribution corresponding with the distribution of this second type of cell (Fig. 3). Finally a few non-lymphoid cells (not restricted to the granulomas) were ACP$^+$ and HLA-DR$^-$.

At the PPD test sites, the same three types of non-lymphoid cells were seen but their relative proportions were different (Table 4). The type II RFD1$^+$, ATPase$^+$, HLA-DR$^+$ cells were more numerous than the type I RFD-2$^+$, ACP$^+$, HLA-DR$^+$ macrophage like cells. Only a few type III HLA-DR$^-$, ACP$^+$ macrophages could be detected. In the positive Kveim reaction the RFD1$^+$, ATPase$^+$ non-lymphoid cells (type II) were often seen in the centre of the granulomas. Here these type II cells were seen in association with T4$^+$ cells. Only a few T8$^+$ cells were detected in the close vicinity. In the Kveim test site Type II cells were slightly more numerous than the type I RFD-2$^+$, HLA-DR$^+$, ACP$^+$ macrophage like cells.

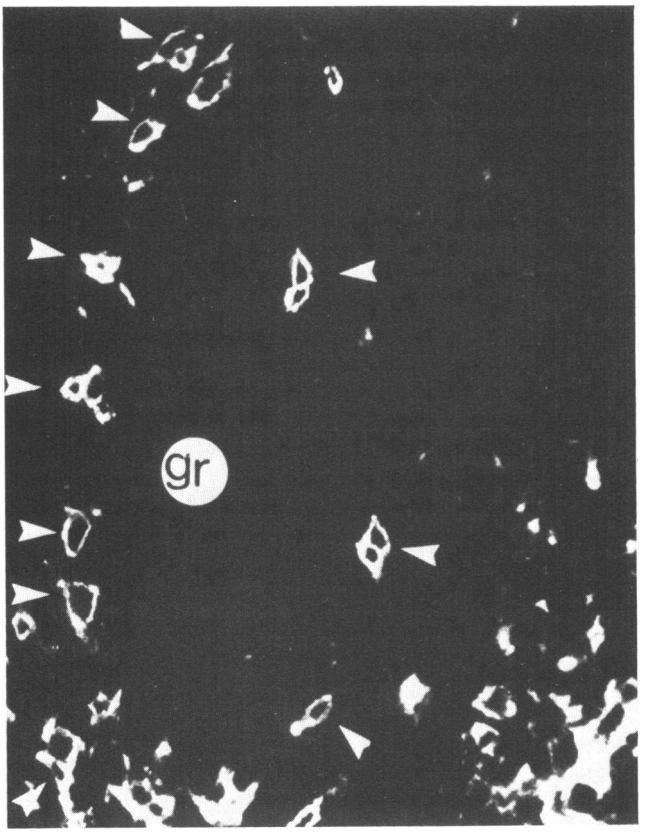

Fig. 3. Sarcoid granuloma stained with RFD-1, an antibody specific for dendritic or interdigitating cells. Cells at the periphery of the granuloma (arrows) are RFD-1$^+$ and correspond to the strongly HLA-DR$^+$, ACP$^-$ cells.

In the final part of the study, cells with the features of Langerhans cell (LCs) were analysed. None of the HLA-DR$^+$, ATPase$^+$ cells in the sarcoid granuloma, Kveim or PPD test sites were NA1/34$^+$, thus excluding the possibility that these cells were Langerhans cells. Some cells ($< 3\%$) with the phenotype of LCs were seen in the dermis of biopsies taken from sarcoid lesions but these were scattered around rather than constituting an integral part of the granuloma. Their phenotype was HLA-DR$^+$, NA1/34$^+$, ATPase$^+$.

In certain areas of the sarcoid skin biopsies there was a significant increase in the numbers of LCs in the epidermis. There were 27 LCs per 100 basal layer cells in the diseased areas while normal skin, on average, has only 12 Lcs per 100 basal cells. Increased numbers of LCs were also seen at sites of Kveim tests. Interestingly many LCs were seen in PPD skin test sites of the sarcoidosis patients. The number of these LCs appeared to be in the normal range when compared to normal positive PPD reactions (Poulter *et al.*, 1982).

DISCUSSION

We have shown that the centre of sarcoid granulomas are predominantly composed of strongly HLA-DR$^+$ macrophage like cells and confirm previous studies which showed that macrophages predominate in the sarcoid granuloma (Bjerke, Krogh & Matre, 1981). In addition we have shown that these activated cells retained reactivity with a monocyte–macrophage specific monoclonal antibody RFD-2 as well as strong lysosomal enzyme (ACP) activity. Immunofluorescent membrane staining of cells in the middle of the granulomas failed to show distinct cell borders (Fig. 2a). The intertwined surface processes that are known to develop in activated macrophages and epithelioid cells (Papadimitriou, Finlay-Jones & Walters, 1973; Adams, 1976) would account for the blurring of cytoplasmic borders.

Interestingly, the periphery of sarcoid granulomas contained an additional population of non-lymphoid HLA-DR$^+$, cells that exhibited only small amounts of ACP activity. The positive identification of these cells was possible because of the strong reactivity of the HLA-DR$^+$ macrophage like cells in this area with another antibody RFD-1, which in contrast to RFD-2, recognises HLA-DR$^+$ interdigitating cells in the paracortical T cell area of nodes dendritic cells in the kidney (Raftery *et al.*, 1983) as well as in the thymic medulla (Bofill, M. & Janossy, G. personal communication). An additional identity tag for these cells was their ATPase activity. On the basis of this phenotypic analysis we would like to suggest that the central and peripheral parts of the well developed granulomas have different characteristics. In the centre, activated macrophages, and 'epithelioid cells' identified on morphological grounds appear to be strongly HLA-DR$^+$. This finding is consistent with the view that these cells derive from the macrophages (Boros, 1978). Thus the heterogeneity of DR positivity on giant cells (Rowe *et al.*, 1981; Thomas *et al.*, 1982) may reflect differences in origin, or the presence of certain cell types (e.g. infiltrating T cells in the lesion) that stimulate DR$^+$ expression on other cells. In the periphery the HLA-DR$^+$ cells correspond to the features of antigen presenting cells (Balfour *et al.*, 1981; Poulter, 1983). The occasional RFD-2$^+$, HLA-DR$^-$ cells that have negligible acid phosphatase lysosomal enzyme (type 3 cell in Table 4), are likely to be recently arrived monocytes that have not been activated into macrophages.

Analysis of T cell subsets in the sarcoid granulomas also reveals a significant differential localization. The T4$^+$ cells of the inducer type are dominant in the middle of the granuloma around the DR$^+$ macrophage like cells. In contrast the T8$^+$ cells are found only in the periphery where they are admixed with T4$^+$ cells. These findings agree with other authors' observations about the distribution of lymphocytes in sarcoid lesions in other tissues (Semenzato *et al.*, 1982; Modlin *et al.*, 1983). This kind of distribution of lymphocytes in granulomas is not specific for sarcoidosis but reflects a more general pattern that has also been recorded in the tuberculoid forms of leprosy but not in the lepromatous form (Narayan *et al.*, 1983; Modlin *et al.*, 1983). One would therefore like to suggest that the lack of suppressor/cytotoxic influences in the deep areas of the granulomas in the presence of many HLA-DR$^+$ macrophages and T4$^+$ cells of the inducer type may contribute to the chronicity of these lesions. One difference between our findings in the skin and the observation in bronchoalveolar lavage (Crystal *et al.*, 1981) is the higher proportion of macrophages in the lesions

in the skin and the low proportion of macrophages in the lavage fluid. We think that this may be due to selective loss of macrophages during the collection of the lavage fluid especially because our own observation on active sarcoid lung lesions obtained by transbronchial lung biopsy (unpublished) and those of others (Semenzato, personal communication) are that the proportion of macrophage like cells is higher than lymphoid cells. Thus the observations in skin are likely to reflect a general pattern in granulomas in all systems.

The distribution of $T4^+$ cells, $T8^+$ cells and other non-lymphoid cells in the granulomas of the positive Kveim test is similar to the sarcoid lesion. The occasional observation of cells with the RFD-1^+ phenotype of interdigitating (dendritic) cell in the centre of the small epithelioid nodules in the Kveim granulomas contrasts with the finding in long standing lesions of sarcoid patients. This suggests that the granulomas start around these interdigitating type cells. Indeed, it has been shown that the growth in cell numbers occurs mainly by new arrivals on the periphery of recently induced experimental granulomas (Ryan & Spector, 1969).

Although the number of PPD skin test sites and Kveim biopsies studied is small, the study addresses itself to three of the possible mechanisms by which negative PPD reactions may occur: (a) lack of antigen presenting cells; (b) excess of suppression and (c) deficiency of inducer influence. As we have shown that at the site of the negative PPD reaction many Langerhans cells are present the first possibility is unlikely. However, it is still conceivable that these cells do not generate an inflow of sufficient numbers of lymphocytes. The absence of any dramatic increase in the proportion of $T8^+$ cells at the site of PPD injection makes it unlikely that an excess of suppressor influence is the cause of the negative PPD reaction. However, the low proportion of $T4^+$ cells suggests a relative deficiency of the inducer type immunoregulatory influences. Comparison with the rate of cell arrival at the PPD positive site in a normal individual (Poulter *et al.*, 1982) leads to the speculation that the low proportion of $T4^+$ cells in the sarcoid patients PPD reaction is due to sluggish arrival of $T4^+$ cells to the PPD lesion. In contrast, it is intriguing that there are plenty of $T4^+$ cells in the Kveim reaction (T4:T8 ratios 4:1; see Table 3 & Fig. 1c). As the Kveim reaction takes more time to develop than the PPD reaction the high proportion of $T4^+$ cells at the site of positive Kveim reaction cannot be compared with the PPD reaction. Unfortunately, we do not know the proportion of antigen specific cells in the incoming T cell populations. The tuberculin sensitive T cells of $T4^+$ type may be depleted or sequestered while the $T4^+$ cells sensitized to Kveim antigens due to sarcoidosis may be increased. Thus it is possible that unhindered accumulation of $T4^+$ cells around large HLA-DR^+ macrophage like cells of the sarcoid lesion leads to altered circulation or decreased availability of PPD specific $T4^+$ cells.

In comparison to the sarcoid skin lesion, the granulomas in a positive Kveim reaction appear to have the same type of cells in a similar distribution. We would therefore suggest that the Kveim reaction is a suitable model of granuloma formation in sarcoidosis.

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