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MEMBRANE RECEPTOR SITES FOR THE IDENTIFICATION OF LYMPHORETICULAR CELLS IN BENIGN AND MALIGNANT CONDITIONS

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Summary.—The cells of the lymphoreticular system are heterogeneous both morphologically and functionally. The bone marrow derived (B) lymphocyte can be identified by the presence of easily detectable surface immunoglobulin and a receptor for antigen–antibody–complement (EAC) complexes. Monocytes and histiocytes also bear a receptor for EAC and in addition possess a receptor for cytophilic antibody detected with red cell—IgG complexes (IgGEA). In man, thymus derived (T) lymphocytes form non-immune rosettes with sheep red blood cells (E). We have examined a number of malignant lymphoreticular populations for the presence of the EAC, EA, and E receptors on suspensions of cells and have adapted the technique to demonstrate the EAC and EA receptors on frozen tissue sections. Rosetted malignant cells can also be cytologically examined on Millipore filters. The malignant cells both in section and suspension from the spleens and lymph nodes of 6 patients with nodular lymphoma bound EAC but not IgGEA or E; by these criteria these malignant cells are of B lymphocytic origin. The malignant cells from the spleens of 2 patients with leukaemic reticuloendotheliosis and 1 patient with malignant histiocytosis could be classified as being of histiocytic origin by the selective binding of IgGEA. In 3 cases of diffuse lymphocytic lymphoma the malignant cells bound only E and are therefore of T lymphocytic origin. The application of these techniques to the classification of malignant lymphoma may lead to important theoretical and therapeutic advances.

THE TRADITIONAL approach to the classification of malignant lymphomata has been based predominantly upon morphology. A neoplastic cell has been named for the benign lymphoreticular cell that it most resembles. This approach has led to classifications which are useful clinically and pathologically. However, recent immunological advances have demonstrated a greater heterogeneity among lymphoreticular cells than previously thought. For example, cells morphologically classified as lymphocytes consist of T cells (thymus dependent lymphocytes), B cells (thymus independent lymphocytes) and probably also stem cells. Recently, a number of different surface markers have been identified on these different lymphoreticular cells. B cells can be identified by the presence of easily detectable surface immunoglobulin (Raff, 1970). Most B cells also have receptors for the third component of complement (C3) and can be identified by their binding of red cells coated with antibody and complement (EAC) (Bianco, Patrick and Nussenzweig, 1970). B lymphocytes also bear a receptor for the Fc portion of the immunoglobulin molecule, demonstrable with radio-labelled soluble antigen–antibody complexes (Basten *et al.*, 1972) or with fluorescein labelled aggregated human IgG (Dickler and Kunkel, 1972). Most monocytes, histiocytes and macrophages also bear C3 receptors (Huber *et al.*, 1968), but can be distinguished from B lymphocytes in that they have in addition receptors for cytophilic antibody

(Berken and Benacerraf, 1966) and will bind red cells coated with IgG (IgGEA) (Huber, Douglas and Fudenberg, 1969). Receptors for IgG and for C3 can also be identified on cells in frozen tissue sections as well as on cells in suspension (Dukor, Bianco and Nussenzweig, 1970; Shevach, Jaffe and Green, 1973). Human T cells bear none of these receptors, but living cells in suspension can be identified by their ability to form non-immune rosettes with sheep red blood cells (Lay *et al.*, 1971; Jondal, Holm and Wigzell, 1972). Recent studies indicate that these receptors can also be identified on neoplastic lymphoreticular cells, and it is suggested that their presence or absence may indicate the origin of the neoplastic cells. In this report we describe the investigation of a number of different lymphoreticular tumours for the presence of the C3, IgG, and E receptors both in cell suspensions and on frozen sections where applicable. We also describe the distribution of cells bearing these receptors in normal lymphoreticular tissues used as controls.

MATERIALS AND METHODS

Preparation of cell suspensions and frozen sections.—Immediately after surgical removal, a small portion of the spleen or lymph node was minced finely in RPMI-1640 (Grand Is. Biological Co.) with 10% foetal calf serum. The minced tissue and supernatant were then filtered through stainless steel wire mesh. A pellet was obtained and the red cells were lysed with ammonium chloride. In some instances spleen suspensions containing large numbers of granulocytes were purified on a Ficoll (Pharmacia)–Hypaque (Winthrop Labs) gradient, and the mononuclear cell layer isolated. The white cell suspensions were washed 3 times in serum-free medium and brought to a concentration of $2{\cdot}5 \times 10^6$/ml. For studies of receptors on cells in tissue sections, representative tissue blocks were frozen immediately in O.C.T. embedding medium (Ames Co.) and maintained at — 70°C until sectioning. The remainder of the tissue was fixed and processed in a conventional manner and used for definitive histopathological diagnosis.

Rosette assay for the binding of IgMEAC and IgGEA in suspensions and frozen sections.—Rabbit IgG and IgM anti-Forssman antibodies to sheep red blood cells (SRBC) were prepared as previously described (Frank and Gaither, 1970). They were isolated by gel filtration on Sephadex G-200 columns followed by sucrose gradient ultracentrifugation. Sensitized sheep erythrocytes (EA) were prepared with isolated rabbit IgG and IgM antibodies by using the optimal concentration of antibody for maximal complement mediated haemolysis at 37°C. To prepare IgMEAC complexes, normal mouse serum was used as the source of complement (C). It was diluted 1 : 10 in veronal buffered saline containing optimal concentrations of Ca^{++} and Mg^{++} in 0·1% gelatine (VBS) and then added to an equal volume of IgMEA (5×10^8/ml in VBS) and incubated at 37°C for 30 min. The cells (IgMEAC) were then washed twice in phosphate buffered saline (PBS) and reconstituted to the concentration described below. It should be noted that IgGEAC is an unsuitable reagent and is not used because IgGEA sites within the EAC complex would also bind to histiocyte IgGEA receptors. A receptor for heterologous IgM has not been identified on human cells (Huber and Fudenberg, 1968). Thus, IgMEA should not bind to any cell and serves as a control reagent for nonspecific binding of red blood cells.

Equal volumes of the white cell suspensions ($2–3 \times 10^6$/ml) and the IgGEA, IgMEAC or IgMEA suspensions (1×10^8/ml) were mixed in plastic tubes and gently rotated at 37°C for 30 min. An aliquot of the suspension was then mixed with an equal volume of trypan blue and examined in a haemacytometer chamber. Only viable cells were counted and viability of the tissue suspensions was generally greater than 90%. If the final viability was less than 80%, the suspension was discarded. Any cell binding 3 or more erythrocytes was scored as positive. A minimum of 200 cells was counted and the percentage of rosette forming cells determined. It should be noted that conditions producing E rosette formation, *i.e.*, 37°–4°C, were never used and IgMEA, used as a control, was consistently negative under the conditions used in these experiments. For cytological identification of individual rosetted cells, the rosetted cell suspension was fixed

in an equal volume of Perfix (Applied Bioscience). The fixed suspension was then centrifuged and the pellet resuspended in 70% ethanol. An aliquot (0·2 ml) was filtered on to a Millipore membrane (Millipore Corpn) with a pore size of 5 nm, and stained with haematoxylin and eosin.

For rosette formation on tissue sections, 8 μm frozen sections of unfixed tissue were cut with a cryostat and allowed to air dry. The sections were layered with IgGEA, IgMEAC, or IgMEA (1×10^8/ml) and incubated at room temperature for 30 min. The slides were then washed 3 times in phosphate buffered saline to remove non-adherent red cells. The resultant preparations were fixed for 15 min in Perfix, stained with haematoxylin and eosin, and examined by light microscopy.

Rosette assay for the binding of sheep red blood cells (E_N).—A modification of the method of Weiner, Bianco and Nussenzweig (1974) was used. Sheep red blood cells (SRBC) were treated with neuraminidase from *Clostridium perfringens* (Sigma Chemical) and adjusted to a concentration of 1×10^8/ml in RPMI-1640. Equal volumes of the neuraminidase treated SRBC (E_N) and the white cell suspensions ($2–3 \times 10^6$/ml) were mixed and incubated for 15 min at 37°C. The suspensions were then sedimented at room temperature for 10 min at 200 *g*. The supernatant was gently removed and replaced with 100% foetal calf serum which had been previously absorbed with SRBC. After an 18-h incubation at 4°C, the pellet was gently resuspended and the number of viable cells binding 3 or more erythrocytes counted.

RESULTS AND DISCUSSION

The binding properties of the IgMEAC, IgGEA and E_N reagents to the different cell types are shown in Table I. IgMEAC binds to both B lymphocytes and to monocytes/histiocytes. IgGEA binds only to monocytes/histiocytes. It should be noted that the IgGEA reagent we have used does not detect the Fc receptor on B lymphocytes (Shevach *et al.*, 1972), which can best be detected by radiolabelled soluble antigen–antibody complexes (Basten *et al.*, 1972) or by aggregated human IgG (Dickler and Kunkel, 1972). E_N binds only to living T cells and E rosette formation can be seen only in cell suspensions and not on frozen sections (Shevach *et al.*, 1973). It should also be noted that in the frozen section preparations endogenous red cells are lysed by the freezing and thawing process, and thus do not interfere with interpretation of binding of the added indicator red cells.

TABLE I.—*Rosette Forming Properties of Human Cells*

	IgMEAC	IgGEA	E_N
B lymphocyte	+	0	0
T lymphocyte	0	0	+
Monocyte/histiocyte	+	+	0

Normal lymph nodes and spleens

Control spleens and lymph nodes, histologically normal, were obtained at surgery from patients with a variety of diseases other than malignant lymphoma. The proportion of rosette forming cells in suspensions of control spleens and lymph nodes is summarized in Table II. Briefly, in control spleens the number of complement receptor lymphocytes [CRL

TABLE II.—*Rosette Forming Cells in Control Spleens and Lymph Nodes* (%)

Patient No.	Tissue	IgMEAC	IgGEA	E_N
1	Spleen	33	2	ND*
2	Spleen	37	2	40
3	Spleen	50	13	42
4	Spleen	22	5	ND
5	Spleen	51	11	33
6	Spleen	52	11	39
7	Spleen	41	2	50
8	Spleen	43	8	50
	$\bar{M}$	41	7	42
9	Lymph node	38	0	ND
	Lymph node	19	0	ND
10	Lymph node	43	5	ND
11	Lymph node	41	0	ND
12	Lymph node	33	0	40
13	Lymph node	29	3	66
	Lymph node	36	2	68
14	Lymph node	18	0	82
15	Lymph node	14	0	69
16	Lymph node	43	0	34
	$\bar{M}$	31	1	60

* Not determined.

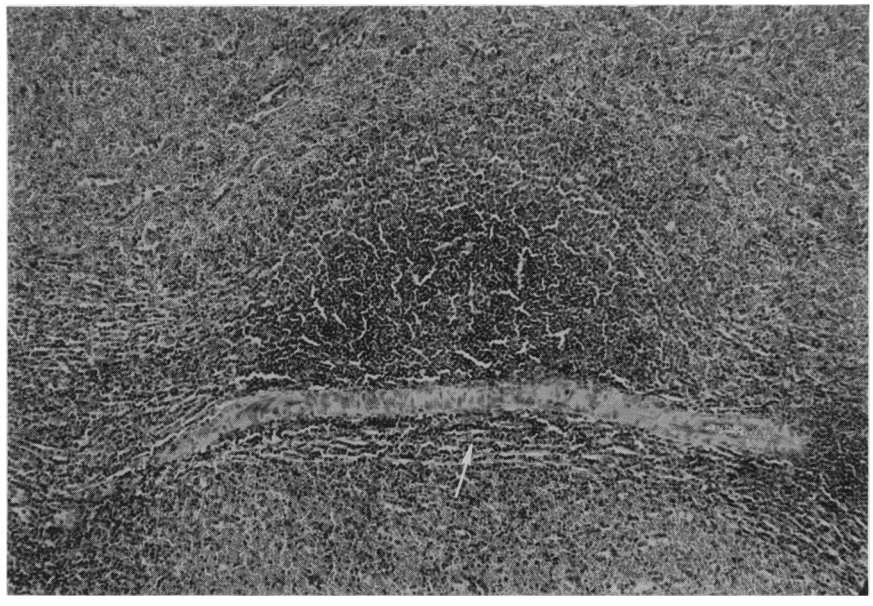

FIG. 1.—Paraffin embedded section of control spleen demonstrating normal splenic architecture. White pulp (lymphocytes) is composed of both periarterial lymphatic sheath (arrow) along central artery as well as eccentric lymphoid follicle. Red pulp (histiocytes) is peripheral. H. & E. × 63.

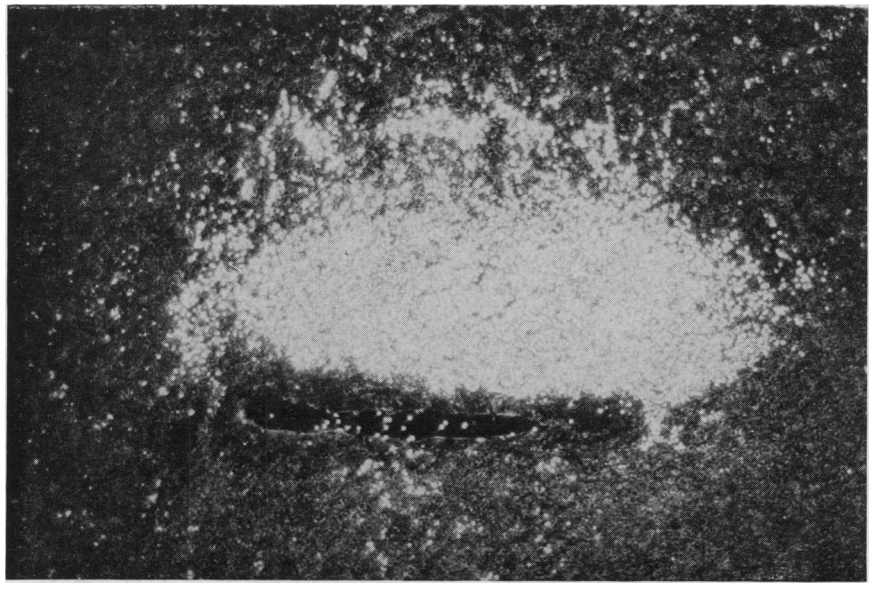

FIG. 2.—Frozen section of spleen seen in Fig. 1 treated with IgMEAC; darkfield microscopy. Reagent red cells appear as white dots and adhere to eccentric lymphoid follicle (B cells). Periarterial lymphatic sheath (T cells) is spared. H. & E. × 63.

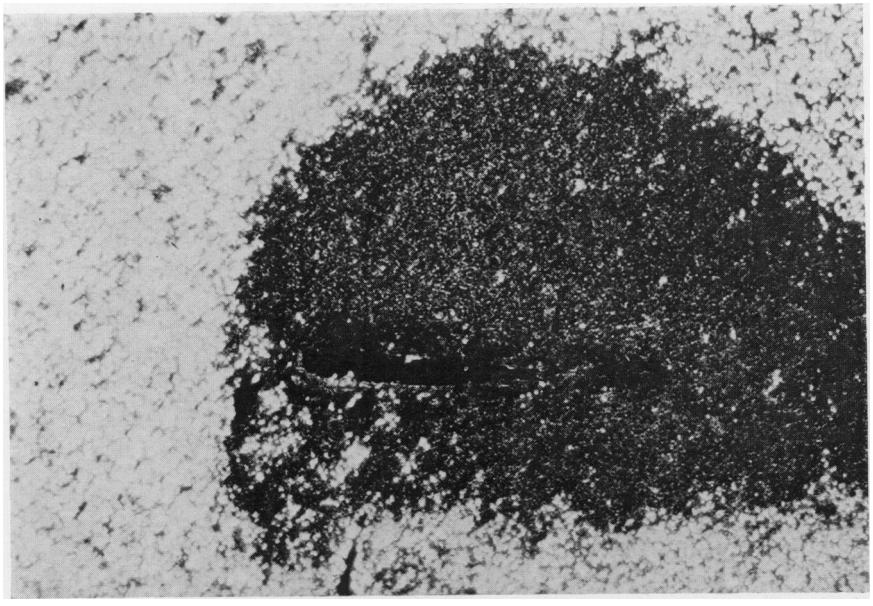

FIG. 3.—Adjacent frozen section to that seen in Fig. 2 treated with IgGEA; darkfield microscopy. Reagent red cells adhere to red pulp (histiocytes) and spare white pulp (lymphocytes) H. & E. × 63.

(B cells)] and the number of E rosette forming lymphocytes [ERFL (T cells)] was approximately equal with means of 41 and 42% respectively. In contrast, the lymphocyte population of lymph nodes tended to be more analagous to that of peripheral blood (Shevach *et al.*, 1972) with a predominance of T cells (ERFL—mean 60%) over B cells (CRL—mean 31%). The number of cells rosetting with IgGEA (histiocytes) tended to be low in suspensions of both organs with a mean of 7% in control spleens and 1% in control lymph nodes. These low figures probably reflect entrappment of histiocytes in the reticulin framework of both spleens and lymph nodes, and an inability to get these cells into suspension.

In frozen sections of control lymph nodes and spleens treated with IgMEAC, the reagent red cells were always observed to adhere to the lymphoid follicles (Fig. 1, 2 and Fig. 4, 5). The reaction tended to be strongest in germinal centres and somewhat less intense in the small lymphocytes of the lymphoid cuff. However, in extremely hyperplastic germinal centres the binding of IgMEAC within the reaction centres was often weak. It is speculated that antigen–antibody–complement complexes bound *in vivo* via the C3 receptor may have blocked the subsequent reaction with the IgMEAC reagent. The C3 receptor is thought to play a role in the follicular localization of antigen (Nussenzweig and Pincus, 1972). Furthermore, Nussenzweig and Pincus speculate that the interaction of immune complexes with follicular B lymphocytes may also contribute to an increase in immunoglobulin synthesis during an antibody response (1972). Thus, the presence of large numbers of complexes within a hyperplastic germinal centre would not be unexpected.

The C3 receptor appears to be a constant and characteristic feature of follicular B lymphocytes. In lymph nodes, although immunoglobulin bearing B cells are thought to be distributed in both follicles and medullary cords (Craddock, Longmire and McMillan, 1971), it appears

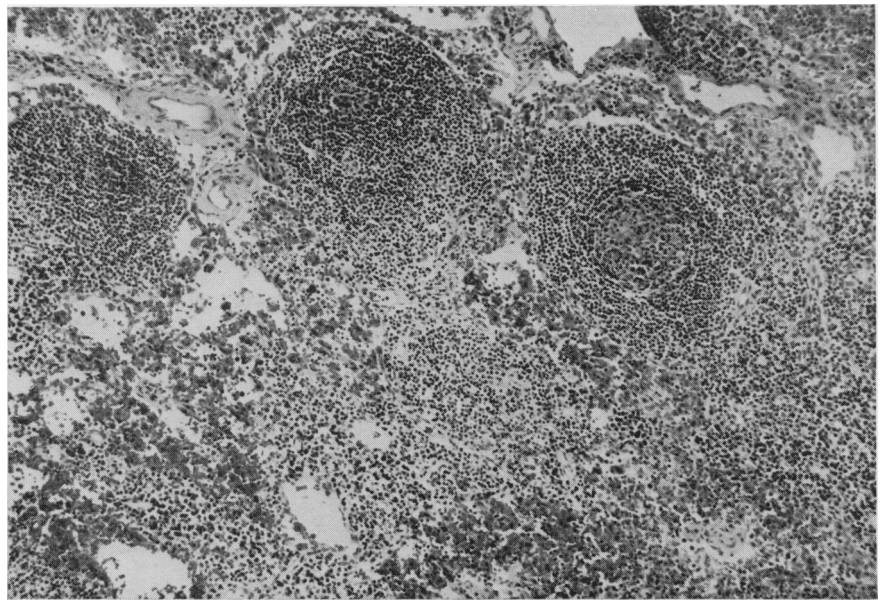

FIG. 4.—Paraffin embedded section of control lymph node showing primary and secondary follicles. Cortical and medullary sinuses are dilated and show sinus histiocytosis. H. & E. × 63.

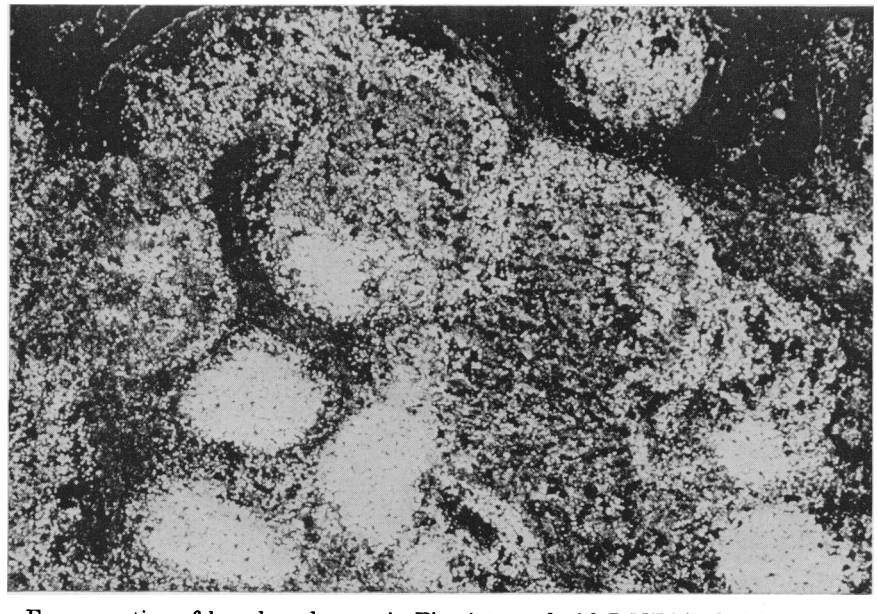

FIG. 5.—Frozen section of lymph node seen in Fig. 4 treated with IgMEAC; darkfield microscopy. Reagent red cells adhere to lymphoid follicles (B cells) as well as to histiocytes in cortical and medullary sinuses. H. & E. × 25.

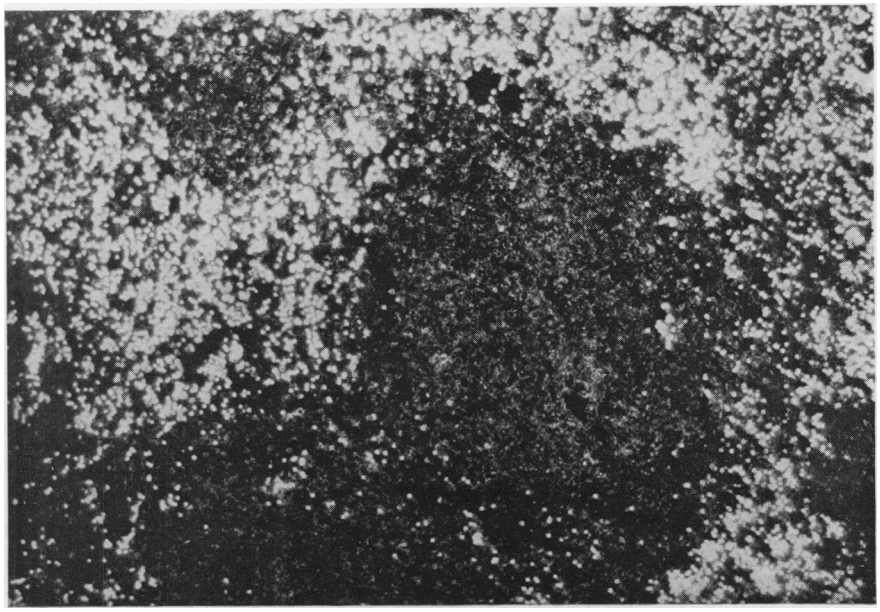

FIG. 6.—Frozen section of lymph node demonstrating sinus histiocytosis, treated with IgGEA; dark-field microscopy. Reagent red cells adhere to histiocytes in dilated sinus but spare lymphocytes in prominent follicle. H. & E. × 63.

that only the cells of the lymphoid follicle demonstrate a C3 receptor with these reagents. One possible explanation for the absence of CRL in the medullary cords is that this area is populated by plasma cells and lymphocytes presumably differentiating to plasma cells. Indeed Bianco *et al.* (1970) pointed out in their initial description of CRL that cells transformed into immunoglobulin producing cells or plasma cells lose the C3 receptor, although it is not known at what stage of B cell differentiation the receptor is lost. Furthermore, B cells at different stages of differentiation may differ quantitatively in the number of C3 receptors and thus exhibit variable binding capacities with these reagents, especially in the frozen section preparations. In the spleen, unlike lymph node, the B cell population may be restricted almost entirely to lymphoid follicles (Craddock *et al.*, 1971). And indeed, Ross *et al.* (1973) have suggested that all surface immunoglobulin bearing cells in human spleen bear the C3 receptor. Such CRL in our material were always localized to follicles. Nussenzweig and Pincus (1972) have speculated that it is the C3 receptor itself that leads to the aggregation of B lymphocytes into follicles *via* formation of a molecular bridge by C3 containing antigen–antibody complexes between the C3 receptor of the B cell and a presumed C3 receptor on the dendritic reticulum cell.

As stated above (Huber *et al.*, 1968), monocytes and histiocytes in peripheral blood also bear C3 receptors. In frozen sections of lymph nodes treated with IgMEAC the reagent red cells, in addition to binding to follicular areas, also bound to the histiocytes in the cortical and marginal sinuses (Fig. 4, 5). These cells, as expected, also bound IgGEA (Fig. 6). By contrast, in spleen, although cells in the marginal zone bound IgMEAC, histiocytes located in the cords of Billroth of the red pulp failed to bind the IgMEAC reagent (Fig. 1, 2). With the IgGEA reagent cells in both the marginal zone and the red pulp were demonstrated

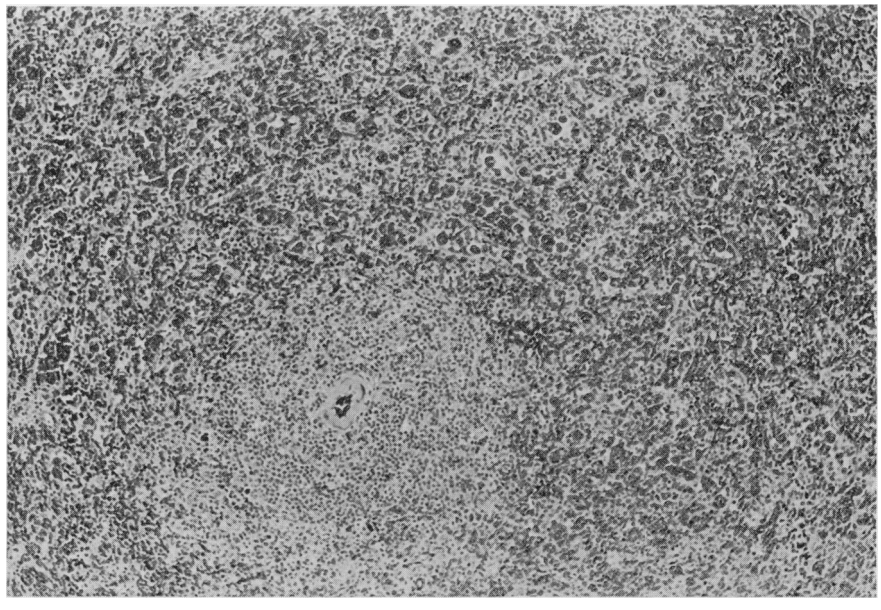

FIG. 7.—Paraffin embedded section of spleen (patient R.P.) involved by histiocytic medullary reticulosis. Proliferating histiocytes in expanded red pulp are engorged with ingested erythrocytes. White pulp is atrophic. H. & E. × 63.

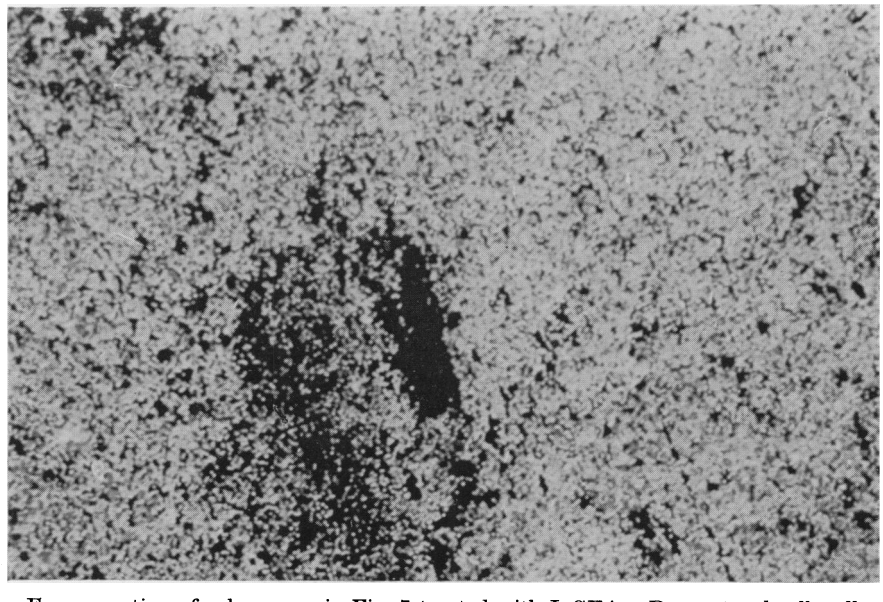

FIG. 8.—Frozen section of spleen seen in Fig. 7 treated with IgGEA. Reagent red cells adhere to neoplastic histiocytes in both red and white pulp areas.

(Fig. 3). The marginal zone of the spleen contains large numbers of macrophages in a reticular mesh receiving a rich arterial supply. It is in this area that antigen, after a brief circulation through the red pulp, is concentrated (Nossal *et al.*, 1966). Since the C3 receptor is thought to play a role in antigen localization, it is not surprising to find avid binding of the IgMEAC reagent to the cells in the marginal zone. Furthermore, the apparent absence of the C3 receptor on histiocytes of the cords of Billroth is less surprising when one considers the relative absence of antigen binding by the red pulp. One must also consider the possibility that these two histiocytic populations of the spleen may differ quantitatively in the number of C3 receptors, and thus exhibit different patterns of reactivity with the IgMEAC reagent. An alternative possibility is that C3 receptors on different cells may show specificity for complement of different species or for different subcomponents of C3. One must note that these studies employed rabbit antibodies and mouse serum as a source of complement. In any event, the basic observations would remain, that these different histiocytic populations consistently exhibit different patterns of reactivity with these reagents. More detailed studies are in progress to resolve these questions.

Pathological conditions

Histiocytic medullary reticulosis (HMR) as described by Scott and Robb-Smith (1939) is considered the prototype of neoplastic histiocytic proliferations. The neoplastic cells exhibit functional as well as morphological features of histiocytes, as exemplified by their florid phagocytic activity. In one case studied a spleen was obtained from a 19-year old white male who had presented with fever, anaemia and thrombocytopenia. The spleen was enlarged (450 g) and exhibited the classic histopathological features of HMR (Fig. 7). The red pulp was markedly expanded and the white pulp compressed, atrophic and partially obliterated. Both red and white pulp regions were diffusely infiltrated by neoplastic histiocytes, many exhibiting florid erythrophagocytosis. In the cell suspension prepared from the spleen, 53% of the cells bound IgGEA (control: M 7%). Only 19% of the cells bound IgMEAC (control: M 41%), correlating with the reduced lymphocytic population observed histologically. E rosette formation was not studied. In the frozen section preparations of both the spleen (Fig. 8) and a lymph node obtained at autopsy, the neoplastic cells bound IgGEA but failed to bind IgMEAC. Thus, the neoplastic cells demonstrated only one of the receptors of normal histiocytes, that for IgGEA. Of additional interest was that in some areas the neoplastic cells appeared to " dedifferentiate " both morphologically and functionally, in that they appeared cytologically more atypical and failed to demonstrate phagocytosis. However, these cells still bound IgGEA, apparently retaining their histiocytic marker.

Leukaemic reticuloendotheliosis or " hairy cell leukaemia " is an entity in which the origin of the neoplastic cells has been in dispute, largely because of their apparent hybrid nature. The cells appear to have features of both lymphocytes (Rubin *et al.*, 1969) and monocytes or histiocytes (Yam *et al.*, 1968). The spleens of 2 patients with leukaemic reticuloendotheliosis were obtained for study (Jaffe *et al.*, 1974*a*) and were replaced almost entirely by neoplastic cells. In cell suspension as well as in frozen sections, the neoplastic cells bound IgGEA but failed to bind IgMEAC or E (Table III). Thus, these neoplastic cells demonstrated the receptor for cytophilic antibody characteristic of histiocytes or monocytes, and failed to demonstrate any of the markers of either B or T lymphocytes.

Nodular or follicular lymphoma represents another entity of controversial cytogenesis amenable to study by these techniques. Although Gall and Mallory (1942) and Rappaport, Winter and Hicks

TABLE III.—*Rosette Forming Cells in Tissues of Patients with Malignant Lymphoma* (%)

Patient	Diagnosis*	Source†	IgMEAC	IgGEA	E_N
R.P.	HMR	S	19	53	ND
J.S.	LRE	S	4	72	ND
H.G.	LRE	S	4	86	1
B.A.	PDL, N	S	22	5	51
	PDL, N	LN	21	2	43
	PDL, N	LN	18	1	46
D.H.	MLH, N	LN	44	0	38
L.K.	PDL, N	S	45	10	45
	PDL, N	LN	30	2	34
	MLH, N	LN	61	10	36
K.M.	PDL, N	LN	61	1	20
W.M.	PDL, N	LN	39	5	40
A.P.	MLH, N	S	56	2	34
	MLH, N	LN	54	3	54
	MLH, N	LN	43	2	54
P.R.	PDL, D	LN	15	35	71
A.H.	PDL, D	LN	19	10	87
D.J.	PDL, D	LN	9	4	75

* HMR Histiocytic medullary reticulosis.
LRE Leukaemic reticuloendotheliosis.
PDL Malignant lymphoma, poorly differentiated lymphocytic type.
MLH Malignant lymphoma, mixed lymphocytic–histiocytic type.
N Nodular.
D Diffuse.
† S Spleen.
LN Lymph node
ND Not determined.

(1956) defined the histopathological criteria for distinguishing nodular lymphoma from benign follicular hyperplasia, the relationship between the neoplastic nodules and lymphoid follicles has remained uncertain. We have studied the cells from 6 cases of nodular lymphoma (Fig. 9) for the presence of these membrane receptors (Jaffe *et al.*, 1974*b*). The neoplastic cells, both in suspension as observed on the Millipore filter preparations and in frozen sections, bound IgMEAC (Fig. 10) but failed to bind IgGEA or E_N. These binding properties are characteristic of B cells of the lymphoid follicle, as described above. Furthermore, in 3 cases studied subclassified as mixed lymphocytic–histiocytic (mixed small and large cell), both cell types were observed to bind only IgMEAC. These data suggest that both morphological variants are indeed B cells and are derived from the lymphoid follicle.

Whereas nodular lymphoma appears to represent a B cell proliferation, 3 cases of diffuse lymphoma were studied in which the neoplastic cells appeared to be T cells. All 3 cases demonstrated similar histopathological features (Fig. 11). Normal nodal elements were replaced by a diffuse infiltrate composed of atypical lymphocytes admixed with smaller numbers of benign-appearing histiocytes. The histiocytes were often in small clusters and appeared " epithelioid ". The lymphocytes were round to oval with sparse cytoplasm and a wide range of nuclear maturation. Although many appeared almost mature, others had larger, more open nuclei with finely clumped chromatin in a clear parachromatin and one or 2 small nucleoli. Upon examination under oil, many lymphoid cells were observed with multiple deep nuclear grooves. A few neoplastic cells had large irregular vesicular nuclei, finely stippled chromatin, prominent eosinophilic nucleoli and abundant pale cytoplasm. Although these cells resembled mononuclear variants of Reed–Sternberg cells, no classic Reed–Sternberg cells were observed. In suspensions prepared from these lymph

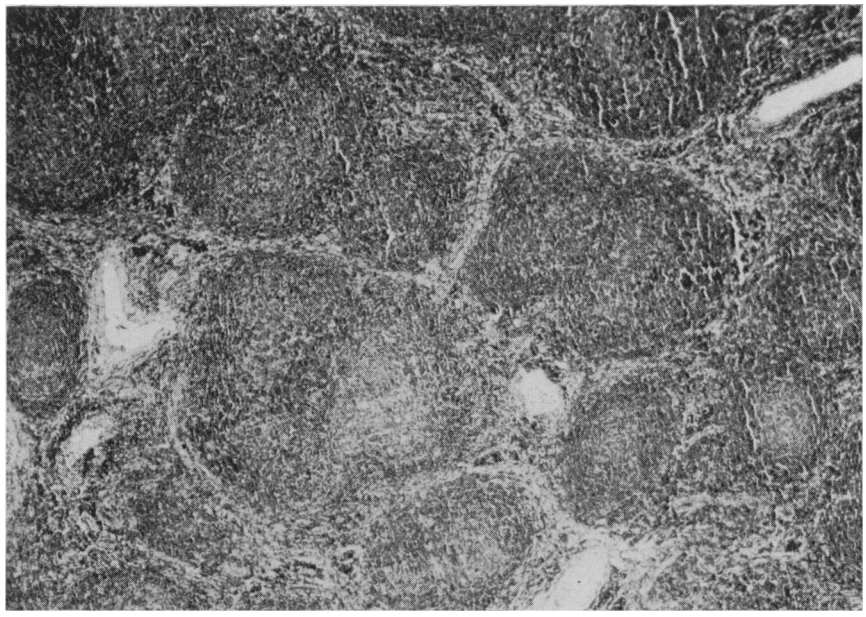

FIG. 9.—Paraffin embedded section of lymph node (patient L.K.) involved by nodular lymphoma, mixed lymphocytic–histiocytic type. Neoplastic nodules composed of a mixture of small and large cells replace the lymph node. H. & E. × 25.

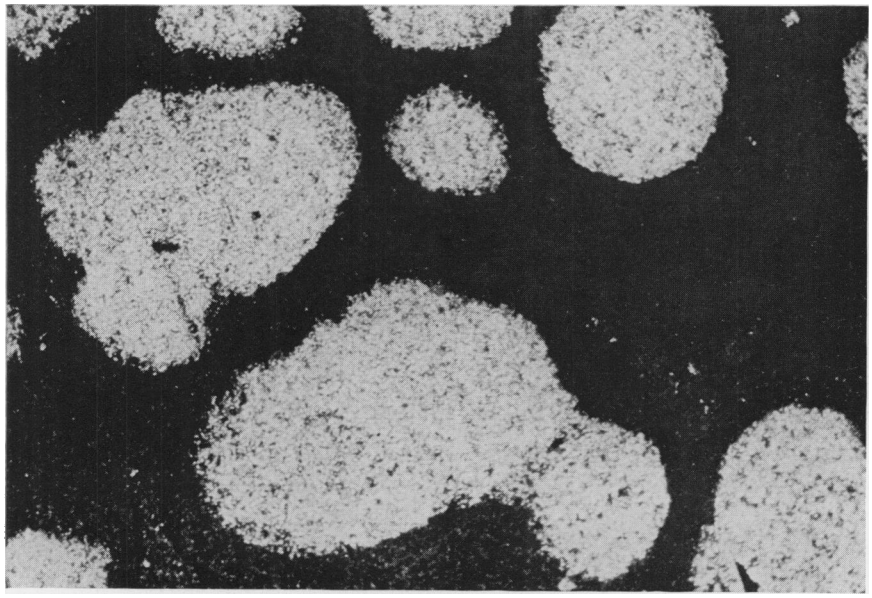

FIG. 10.—Frozen section of lymph node seen in Fig. 9 treated with IgMEAC; darkfield microscopy. Reagent red cells bind diffusely to neoplastic nodules and spare normal appearing lymphocytes in compressed internodular cords. H. & E. × 25.

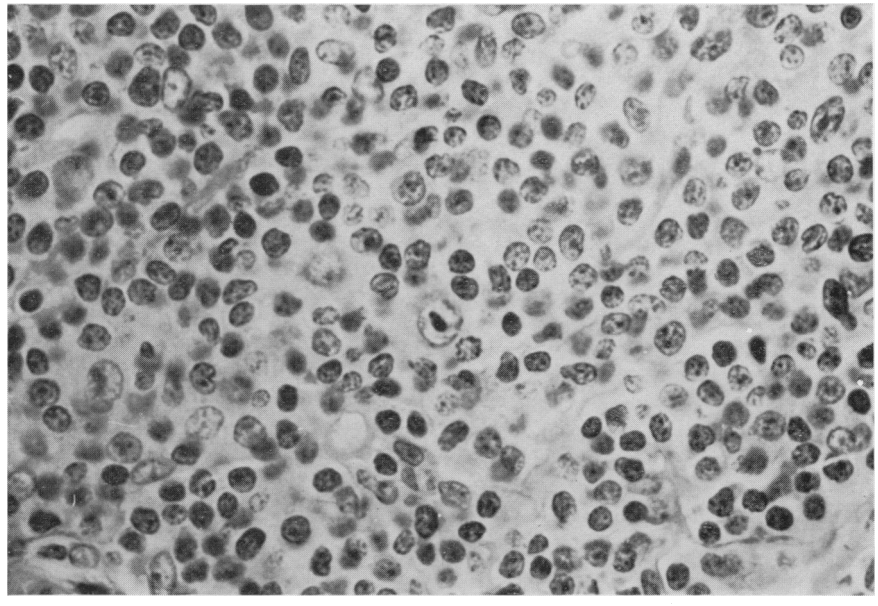

FIG. 11.—Paraffin embedded section of lymph node (patient D.J.) involved by malignant lymphoma, poorly differentiated lymphocytic type, diffuse. Infiltrate is composed of pleomorphic lymphocytes and infrequent large atypical mononuclear cells with prominent nucleoli. H. & E. × 400.

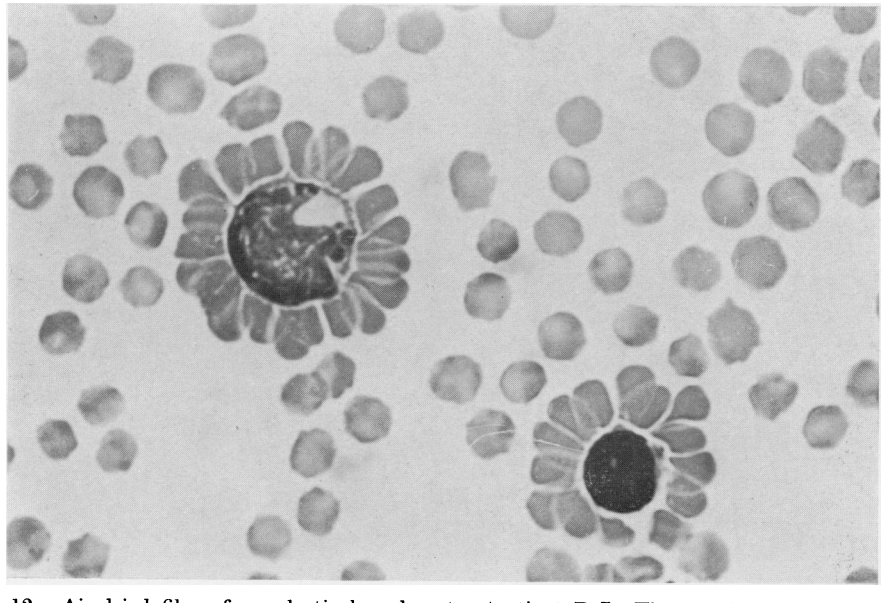

FIG. 12.—Air-dried film of neoplastic lymphocytes (patient D.J., Fig. 11) forming E rosettes. Wright's stain. × 1000.

nodes, a high proportion of the cells bound E_N (Table III). On the Millipore filter preparations these rosetted cells were neoplastic appearing lymphocytes, also demonstrable in air dried films stained with Wright's stain (Fig. 12). The large atypical mononuclear cells, although rare, were also observed to form E rosettes. Only a small proportion of the cells bound either IgGEA or IgMEAC (Table III), and on the Millipore filter preparations these rosetted cells were predominately benign appearing histiocytes. Occasionally small lymphocytes were also observed to form IgMEAC rosettes. Thus, in these 3 cases of diffuse lymphoma the neoplastic cells formed E rosettes and by this criterion were T lymphocytes.

The accumulation of benign appearing histiocytes was an interesting and consistent histological feature in all 3 cases. It is speculated that perhaps migration inhibition factor (MIF), which is known to be a T cell product (David, 1966) was being produced by these neoplastic T cells. Furthermore, such a synthesis by a neoplastic population would probably go unregulated and might be expected to result in the accumulation of histiocytes within the lesion.

Another interesting feature was the clinical history given by one of the above patients (A.H.). Three years before the diagnosis of her lymphoma on an inguinal lymph node biopsy, she had had a thyroidectomy for Hashimoto's thyroiditis. This diagnosis was subsequently confirmed here upon review of the pathological material. Hashimoto's thyroiditis recently has been claimed to be associated with an increased proportion of circulating T cells (Farid *et al.*, 1973). Thus, it would appear that this patient with a T cell lymphoma had evidence of a hyperactive cellular immune system 3 years earlier. Of course, any speculation as to the significance of this finding in relation to the pathogenesis of her lymphoma is conjectural.

In this report we have investigated a number of neoplastic lymphoreticular proliferations for the presence of the IgMEAC, IgGEA and E receptors. It is suggested that the presence or absence of these various receptors may permit classification of the cellular elements as to their origin from B cells, T cells or histiocytes. Two techniques employed in this study are particularly important for identifying the receptors present on the neoplastic cells; these are the Millipore filter technique for examining the rosetted cells and the frozen section technique for the IgMEAC and IgGEA rosettes. Because of the often focal involvement of lymphoid organs by lymphoma and also the polymorphous nature of many infiltrates with admixed benign cellular elements, the suspension data alone cannot give unequivocal results as to the receptors present on the neoplastic cells. Examination of the Millipore filter preparations is crucial for establishing the neoplastic or non-neoplastic identity of the rosetted cells, and examination of the frozen section preparations reveals the distribution of these rosetted cells.

It is hoped that application of the techniques used in this report will lead to advances in the classification of all reticuloendothelial neoplasms and increase our understanding of these diseases. These studies may also have application in the search for aetiologies. For example, recent work suggests that B cells and T cells may differ in their susceptibility to various oncological agents (Haran-Ghera and Peled, 1973). Other theoretical applications are the use of specific receptors as possible target sites in the therapy of malignant lymphomata. Finally, these techniques can also be applied to the identification of mononuclear cells within the lesions of a wide variety of benign immunopathological and inflammatory conditions.

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REFERENCES

BASTEN, A., MILLER, J. F. A. P., SPRENT, J. & PYE, J. (1972) A Receptor for Antibody on B Lymphocytes. I. Method of Detection and Functional Significance. *J. exp. Med.*, **135**, 610.

BERKEN, B. & BENACERRAF, B. (1966) Properties of Antibodies Cytophilic for Macrophages. *J. exp. Med.*, **123**, 119.

BIANCO, C. R., PATRICK, R. & NUSSENZWEIG, V. (1970) A Population of Lymphocytes Bearing a Membrane Receptor for Antigen–antibody Complement Complexes. I. Separation and Characterization. *J. exp. Med.*, **132**, 702.

CRADDOCK, C. A., LONGMIRE, R. & MCMILLAN, R. (1971) Lymphocytes and the Immune Response. Part II. *New Engl. J. Med.*, **285**, 378.

DAVID, J. R. (1966) Delayed Hypersensitivity *in vitro*: its Mediation by Cell-free Substances Formed by Lymphoid Cell–antigen Interaction. *Proc. natn. Acad. Sci. U.S.A.*, **56**,72.

DICKLER, H. B. & KUNKEL, H. G. (1972) Interaction of Aggregated γ-globulin with B Lymphocytes. *J. exp. Med.*, **136**, 191.

DUKOR, P., BIANCO, C. & NUSSENZWEIG, V. (1970) Tissue Localization of Lymphocytes Bearing a Membrane Receptor for Antigen–antibody–complement Complexes. *Proc. natn. Acad. Sci. U.S.A.*, **67**, 991.

FARID, N. R., MUNRO, R. E., ROW, V. V. & VOLPE, R. (1973) Peripheral Thymus-dependent (T) Lymphocytes in Grave's Disease and Hashimoto's Thyroiditis. *New Engl. J. Med.*, **288**, 1313.

FRANK, M. M. & GAITHER, T. (1970) The Effect of Temperature on the Reactivity of Guinea-pig Complement with γ-G and γ-M Haemolytic Antibodies. *Immunology*, **19**, 967.

GALL, E. A. & MALLORY, T. B. (1942) Malignant Lymphoma, A Clinicopathologic Survey of 618 Cases. *Am. J. Path.*, **18**, 381.

HARAN-GHERA, N. & PELED, A. (1973). Thymus and Bone Marrow Derived Lymphatic Leukaemia in Mice. *Nature, Lond.*, **241**, 396.

HUBER, H., DOUGLAS, S. D. & FUDENBERG, H. H. (1969) The IgG Receptor: An Immunological Marker for the Characterization of Mononuclear Cells. *Immunology*, **17**, 7.

HUBER, H. & FUDENBERG, H. H. (1968) Receptor Sites of Human Monocytes for IgG. *Int. Archs Allergy*, **34**, 18.

HUBER, H., POLLEY, M. J., LINSCOTT, W. D., FUDENBERG, H. H. & MULLER-EBERHARD, H. J. (1968) Human Monocytes: Distinct Receptor Sites for the Third Component of Complement and for Immunoglobulin G. *Science, N.Y.*, **162**, 1281.

JAFFE, E. S., SHEVACH, E. M., FRANK, M. M. & GREEN, I. (1974*a*) Leukemic Reticuloendotheliosis: Presence of a Receptor for Cytophilic Antibody. *Am. J. Med.*, **57**, 108.

JAFFE, E. S., SHEVACH, E. FRANK, M. M., BERARD, C. W. & GREEN, I. (1974*b*) Nodular Lymphoma: Evidence for Origin from Follicular B Lymphocytes. *New Engl. J. Med.*, **290**, 813.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface Markers on Human T and B Lymphocytes. I. A Large Population of Lymphocytes Forming Non-immune Rosettes with Sheep Red Blood Cells. *J. exp. Med.*, **136**, 207.

LAY, W. H., MENDES, N. F., BIANCO, C. & NUSSENZWEIG, V. (1971) Binding of Sheep Red Blood Cells to a Large Population of Human Lymphocytes. *Nature, Lond.*, **230**, 531.

NOSSAL, G. J. V., AUSTIN, C. M., PYE, J. & MITCHELL, J. (1966) Antigens in Immunity: Antigen Trapping in the Spleen. *Int. Archs Allergy*, **29**, 368.

NUSSENZWEIG, V. & PINCUS, C. (1972) C3-Receptor Sites on Leukocytes. Possible Role in Opsonization and in the Immune Response. In *Contemporary Topics in Immunobiology*, Vol. 1. Ed. M. G. Hanna Jr. New York–London: Plenum Press, p. 69.

RAFF, M. C. (1970) Two Distinct Populations of Peripheral Lymphocytes in Mice Distinguishable by Immunofluorescence. *Immunology*, **19**, 637.

RAPPAPORT, H., WINTER, W. J. & HICKS, E. B. (1956) Follicular Lymphoma. A Re-evaluation of its Position in the Scheme of Malignant Lymphoma Based on a Survey of 253 Cases. *Cancer, N.Y.*, **9**, 792.

ROSS, G. D., RABELLINO, E. M., POLLEY, M. J. & GREY, H. M. (1973) Combined Studies of Complement Receptor and Surface Immunoglobulin Bearing Cells and Sheep Erythrocyte Rosette-forming Cells in Normal and Leukemic Human Lymphocytes. *J. clin. Invest.*, **52**, 377.

RUBIN, A. D., DOUGLAS, S. D., CHESSIN, L. N., GLADE, P. R. & DAMESHEK, W. (1969) Chronic Reticulolymphocytic Leukemia. *Am. J. Med.*, **47**, 149.

SCOTT, R. B. & ROBB-SMITH, A. H. (1939) Histiocytic Medullary Reticulosis. *Lancet*, ii, 194.

SHEVACH, E. M., HERBERMAN, R., FRANK, M. M. & GREEN, I. (1972) Receptors for Complement and Immunoglobulin on Human Leukemic Cells and Human Lymphoblastoid Cell Lines. *J. clin. Invest.*, **51**, 1933.

SHEVACH, E. M., JAFFE, E. S. & GREEN, I. (1973) Receptors for Complement and Immunoglobulin on Human and Animal Lymphoid Cells. *Transplantn Rev.*, **16**, 3.

WEINER, M. S., BIANCO, C. & NUSSENZWEIG, V. (1973) Neuraminidase Enhancement of the Binding of Sheep Erythrocytes to Human T Lymphocytes. *Blood*, **42**, 939.

YAM, L. T., CASTOLDI, G. L., GARVEY, M. D. & MITUS, W. J. (1968) Functional Cytogenetic and Cytochemical Study of the Leukemic Reticulum Cells. *Blood*, **32**, 90.