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Characterization of cold reactive lymphocytotoxic antibodies in malaria

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SUMMARY

Characterization of cold reactive lymphocytotoxic antibodies present in sera from Thai adults with malaria revealed that the antibodies are predominantly 19S (IgM), directed against both autologous and allogeneic mononuclear cells, complement-dependent, present in titres ranging from 1:2 to 1:16, and exhibit greater lymphocytotoxic activity during the acute stage of malarial infection than during the convalescent stage. The lymphocytotoxic antibodies were primarily directed against B cell targets or both B as well as T cell targets. In addition some sera were reactive with enriched monocyte/macrophage indicator cells at 15°C, but not 37°C. Antibodies directed against B cell targets were lymphocytotoxic both at 15°C as well as 37°C. The results indicate that IgM lymphocytotoxic antibodies in the sera of patients with malaria are directed primarily against B cells with reactivity to a lesser extent against T cells and macrophages and thus may play an immunoregulatory function in the humoral immune response to malaria infection.

INTRODUCTION

We have previously reported the presence of cold reactive anti-lymphocyte antibodies (ALA) in the sera of Thai adults naturally infected with *Plasmodium falciparum* and *Plasmodium vivax* malaria (Wells *et al.*, 1980). ALA were found in 95% of the *P. falciparum* and 98% of the *P. vivax* patients' sera when assayed at 15°C against peripheral blood target cells from uninfected individuals. Furthermore, we have also demonstrated that Thai adults naturally infected with either *P. falciparum* or *P. vivax* have a decrease in both the percentage and absolute number of lymphocytes in general and T lymphocytes in particular (Wells *et al.*, 1979). Finally, in recent studies we have observed that patients with malaria have a loss of functional Con A inducible T suppressor cells and serum suppressor factors (MacDermott *et al.*, 1980).

Cold reactive ALA present in other disease states have been shown to be predominantly IgM and directed against T lymphocytes (Lies, Messner & Williams, 1973; Winfield *et al.*, 1975b). However, cold reactive IgM lymphocytotoxic antibodies directed against B cells also occur in approximately 20% of normal individuals (Park, Terasaki & Bernoco, 1977). Since it is possible that ALA play a role in modulating the immune response of patients with malaria we have further characterized ALA in the sera of malaria patients, with regard to the type of antibody, their relationship to disease activity, the antibody titres and the cell types against which they are directed.

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MATERIALS AND METHODS

Patients. The patient screening process and the collection of sample specimens has previously been described (Wells *et al.*, 1979). In brief, the patients were all out-patients who were mildly ill, recently infected, and not anaemic. Individual patients' peripheral blood was collected in heparinized tubes to obtain mononuclear cells while serum was obtained after allowing a second aliquot of blood to clot in non-heparinized tubes at room temperature.

Isolation of peripheral blood mononuclear cells (MNC) and lymphocyte subpopulations. Peripheral blood MNC were isolated from venous blood centrifuged through Ficoll-Hypaque (Böyum, 1968), then washed three times with Hank's balanced salt solution (HBSS), and resuspended in RPMI 1640 supplemented with L-glutamine, HEPES and 10% heat-inactivated FCS. Initially each sera was screened for the presence or absence of lymphocytotoxic antibodies using autologous, allogeneic patient, and control lymphocytes from healthy donors. Aliquots of the unused sera were then stored at −20°C for use in subsequent assays.

Mononuclear cells from a small number of normal donors were used to determine if the sera lymphocytotoxic antibodies were specific for lymphocyte subpopulations. Mononuclear cells were isolated from a unit of peripheral blood from five healthy Caucasian donors by Ficoll-Hypaque centrifugation. The cells were further separated into mononuclear cell subpopulations (T, B, null cells and macrophages) as previously described (MacDermott & Stacey, 1981). The purity of the resultant cell populations was determined using cell surface characteristics, with values similar to those previously reported (MacDermott & Stacey, 1981).

Lymphocytotoxic antibody assays. The methodology for the lymphocytotoxic antibody assays has been described previously (Wells *et al.*, 1980). Sera with high lymphocytotoxicity from dengue patients or patients with systemic lupus erythematosus served as positive controls while sera from healthy Thai volunteers served as negative controls. Positive sera were subsequently tested for the presence of lymphocytotoxic antibodies using a panel of lymphocytes from 15 normal Caucasian adults of varying HLA phenotype. Reproducibility of the assay checked by repeating the same sera directed against the same targets at different times, demonstrated essentially identical results for the sera tested. Sera with high levels of ALA activity during the initial screenings were diluted serially using commercial pooled human AB sera to determine antibody titres.

Sucrose density fractionation of serum. Sucrose density centrifugation of serum was performed by layering the serum over a 10–40% linear sucrose gradient from which 12 equal fractions were subsequently collected after centrifugation (Kunkel, 1960). Each fraction was tested against normal MNC at 4°C using the microcytotoxicity assay to determine which fractions contained lymphocytotoxic activity. Preliminary experiments were performed to ensure that the sucrose concentration in the various fractions was not cytotoxic to the target indicator cells and did not inhibit ALA in the microcytotoxic assay. All fractions were concentrated to their original volume for use in lymphocytotoxic assays. Quantitative estimation of human IgG, IgM and IgA in the fractions was performed using commercially available regular and low level radial immunodiffusion plates (Hyland Diagnostics, Deerfield, Illinois, USA).

RESULTS

Anti-lymphocyte antibodies (ALA) from 35 sera obtained from *P. falciparum* and *P. vivax* malarious patients during the acute stage of illness were assayed in a microcytotoxicity assay at 15°C against peripheral blood mononuclear cells (MNC) from autologous, allogeneic patient and healthy control donors (Fig. 1). The mean percentage killing by these sera against autologous MNC was 18·3 + 3·5 (mean + s.e.m.), while the mean percentage killing of MNC from allogeneic patients or control donors was 19·7 + 3·7 and 24·6 + 3·8, respectively. Thus, as can be seen in Fig. 1, most malarious patients have antibodies in their sera which are capable of recognizing both autologous as well as allogeneic MNC. When patients' positive ALA sera were serially diluted and tested against allogeneic MNC target cells from healthy Thai donors, the antibodies were noted to be

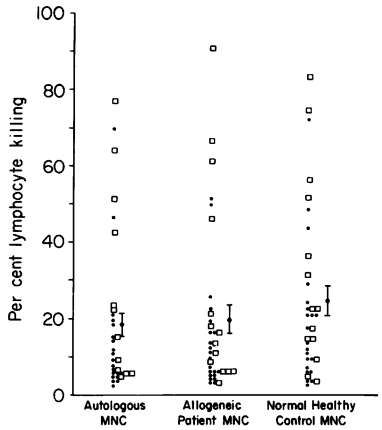

Fig. 1. The ALA of 35 sera from mononuclear cell donor male patients with *P. falciparum* (●) or *P. vivax* (□) malaria tested against autologous and allogeneic target mononuclear cells (MNC) in assays done at 15°C. No ALA (less than 6·5%) were detected when the sera was tested in assays done at 37°C. Sera from healthy male Thai donors was always assayed in parallel with the patients' sera but never demonstrated any significant ALA at 15%C. Percentage lymphocytotoxicity is based on the value after control background for the assay was subtracted.

present in varying titres: 1:2 (40% of samples), 1:4 (30%), 1:8 (20%) and 1:16 (10%). Sixteen of 31 *P. falciparum* (52%) and 19 of 25 *P. vivax* (76%) sera demonstrated high amounts (>40% MNC killed) of cold reactive ALA against peripheral blood MNC targets from 10 healthy Caucasian donors. By contrast, none of 16 sera from healthy controls showed ALA. When a comparison was made on paired antisera collected during both the acute period of illness as well as 15 and 30 days later during the convalescent period, the antibodies generally (11 of 13 ALA positive sera) had higher titres during the acute infection than during the convalescence period (Table 1). Furthermore, six of the seven paired patient sera which were negative for ALA during the acute period did not develop ALA during the convalescent period of illness.

Serum lymphocytotoxic activity was found to be mediated by a protein with the characteristics of an IgM antibody. Treatment with 2-mercaptoethanol followed by iodoacetamide removed or substantially reduced the lymphocytotoxic activity in all the malarious patients' sera. Seven individual sera were examined by sucrose density gradient analysis and the lymphocytotoxicity was consistently found in IgM containing fraction near the bottom of the gradient (tubes 2, 3, & 4 in a 12 tube gradient; Table 2). The lymphocytotoxic activity in the fraction was inhibited by rabbit antiserum specific for human IgM (μ chain). Finally, in four of six individual fractionated sera lymphocytotoxic activity was also found in the IgM containing fraction when assayed at 37°C.

In order to investigate the possibility that IgG or IgA present in fractions 7, 8 and 9 could block IgM-mediated lymphocytotoxicity at either 15°C or 37°C we did 'add-back' experiments. In these experiments we pooled the patients' IgM fractions (2, 3, & 4) as well as the patients' IgG–IgA fractions (7, 8 and 9) respectively. We then added to the IgM fraction equal volumes of either media; autologous patient IgG/IgA; or IgG/IgA fractions from a healthy donor. The IgM fraction of the various patients was equally cytotoxic irrespective of whether it was combined with media, autologous IgG/IgA or IgG/IgA from a normal donor. Thus, IgG/IgA does not inhibit IgM ALA.

Thirty-one sera from patients with acute *P. falciparum* and 25 sera from patients with acute *P. vivax* malaria were tested against T enriched, B enriched and null cell enriched target subpopulation. In a separate group of experiments, patients' sera were tested against monocyte–macrophage enriched (>85% esterase positive) target subpopulations. As seen in Table 3, the lymphocytotoxic antibodies were primarily directed against B cells. Reactivity of ALA against B cells was present both at 37°C as well as at 15°C. In many instances, T cells as well as B cells were killed at 15°C. No reactivity was seen against null cells. Two of six sera showed cold ALA against

Table 1. Lymphocytotoxic activity in malaria patient's sera during acute and convalescent period of illness

Patient	% Parasitaemia	Acute	Days post-admission	
		0	7–15	24–30
P.f.*	1·2	33·5†	0·5	1·5
P.f.	4·2	24·0	1·0	1·5
P.v.	0·05	16·0	0	0
P.f.	0·7	32·5	1·5	0
P.f.	+ve‡	14·5	4·0	3·5
P.f.	+ve	35·5	19·5	19·0
P.f.	4·5	7·5	3·0	2·5
P.f.	0·5	3·0	12·0	27·5§
P.f.	+ve	0	0·5	16·0§
P.f.	+ve	0	8·0	1·0
P.v.	+ve	1	3	0
P.v.	+ve	48·5	33·0	29·5
P.v.	0·04	0	0	5
P.f.	+ve	0	0·5	0
P.f.	0·3	0	3·5	1·5
P.f.	+ve	0	0	0
P.f.	1·2	21·0	9·0	1·0
P.f.	+ve	24·0	12·5	13·5
P.f.	+ve	21·5	22·0	17·5
P.f.	+ve	22·5	20·0	13·5

P.f. = *P. falciparum*: P.v. = *P. vivax*.

* Percentage of donor MNC panel killed by sera. Positive sera = > 40% of donor panel killed. No positive killing of panel MNC by the sera was observed in assays performed at 37°C.

† Average percentage cytotoxic activity in duplicate wells of assays done at 15°C. Assays of individual patients' sera were done in a single experiment using identical target cells.

‡ Parasitaemia verified using Giemsa stained thick slide smear.

§ Indicates that patients experienced relapse or re-infection.

the monocyte–macrophage enriched population, however, no anti-macrophage activity was found at 37°C.

DISCUSSION

We have previously observed that a high percentage of sera from adult Thais with naturally acquired *P. falciparum* and *P. vivax* malaria contained cold reactive lymphocytotoxic activity optimally detected at 15°C (Wells *et al.*, 1980). Because of the association of anti-lymphocyte antibodies (ALA) with dysfunction in immunoregulation in other diseases; and because dysfunctions in the immune response such as decreased T cells (Wells *et al.*, 1979), hypergammaglobulinaemia (Tobie, Abele & Wolff, 1966), decreased Con A inducible suppressor cell capacity and deficiencies in lectin-induced and mitogen-induced cellular cytotoxicity have been found to exist in malarious individuals it is important to characterize cold reactive lymphocytotoxicity to determine the possible *in vivo* significance of ALA during malarial infection in man.

Table 2. Lymphocytotoxic activity in sucrose gradient centrifugation fractions in serum from patients with malaria

Patient	% Parasitaemia†	Temp. (°C)	Percentage lymphocytotoxicity* (fraction number) Neat	F_2	F_3	F_4	F_6	F_7	F_8	F_9
P.v	0·3	15	67·5	32	31	24·5	0	<1	1	0
		37	5·5	18	17·5	13	8	9·5	0	0
P.f.	0·1	15	40·5	4·5	3·0	3·0	1	<1	0	0
		37	0	1	<0	3·0	1	<1	0	0
P.v.	0·1	15	50	26·5	36·5	32·5	<1	0	<1	0
		37	3	8·5	13·0	4·5	0	<1	<1	0
P.f	0·1	15	47·0	35·0	26·0	16·5	1	2	1·5	1
		37	2·5	12·5	12·0	9·5	3·5	1·5	0	2
P.v.	+ve	15	47	40	38·5	35	0	0	0	0
		37	<1	12·5	12·5	9·5	2·5	<1	0	0
P.f.	0·1	15	55·5	38·5	38·0	35·5	0	<1	0	0
		37	1·0	7·5	4·5	3·5	0	1·5	1	1
P.f.	0·7	15	54	31	25	27·5	2·5	0	<1	0
		37	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

P.f. = *P. falciparum*; P.v. = *P. vivax*; n.d. = not done.

* Average cytotoxicity determined from duplicate wells with serum incubated with unfractionated peripheral blood mononuclear cells from healthy Thai adults as described in Materials and Methods. IgM was only detected in fractions 2, 3 and 4 and IgG/IgA was only detected in fractions 7, 8, 9 of the patients serum fractions using radial immunodiffusion plates.

† Percentage parasitaemia determined as described in Table 1.

Table 3. Cell types against which lymphocytotoxic antibodies in malaria sera are directed

Sera	T cells only	B cells only	Both T and B cells	No cells
Normal control (16)*	0†	0	0	100
P. falciparum (31)	3	29	48	19
P. vivax (35)	12	24	44	16

* Number of individual sera examined in parentheses.

† Percentage of sera reacting with T cells only, B cells only, both T and B cells, or no cells.

It is apparent that the cold reactive malaria ALA are similar to cold reactive ALA reported for other disease states (Winfield, Winchester, & Kunkel, 1975a) in that the activity is reduced or eliminated when serially diluted between 1:4 and 1:16. Although no correlation was found between the percentage parasitaemia and the degree of cytotoxicity, it is important to note that cold reactive ALA activity is maintained and gradually declines in serum titre for several weeks following acute malarial infection.

The autocytotoxic antibodies observed in several diseases have been shown to be specific for subpopulations of peripheral blood MNC (Dehoratius & Messner, 1976). However, in view of the occurrence of ALA in a wide variety of human disease states (Ozturk & Terasaki, 1979) it seemed likely that the specificity of malaria related ALA for various MNC subpopulations could be quite

heterogenous. Most reactivity was against B cells particularly at 37°C and to a lesser degree T cells while no anti-null cell activity was found. A number of sera also contained anti-macrophage activity. Thus the primary *in vivo* effect of ALA could be B cell directed in malaria patients.

We have also previously observed that malaria patients' lymphocytes have a normal capacity to respond in the mixed leucocyte reaction (MLR) but a decreased stimulation capability, while a normal response to PHA and Con A is seen (MacDermott *et al.*, 1980). Since stimulatory capacity in the MLR is based on 'LD' or 'Ia' antigens and is different from responding capacity it is possible that IgM anti-B cell antibodies could block Ia antigen recognition leading to suppressed MLR stimulatory capacity.

It is possible that optimal *in vitro* cytotoxicity at 15°C represents a compromise between lower and higher temperature for binding and complement fixation, respectively (Winfield *et al.*, 1975a). *In vivo* and *in vitro* observation on lymphocytotoxins in other human diseases suggest that at 37°C lymphocytes may be able to process lymphocytotoxic antibodies effectively. This has been proposed to result from pinocytosis (Taylor *et al.*, 1971) or shedding of the cytotoxic factor and its receptor (Wernet *et al.*, 1972). At temperatures less than 37°C, which can be seen *in vivo* in the peripheral circulation of patients altered lymphocyte metabolism might impair this process with resultant cell lysis by ALA and interference with normal B cell functional or regulatory capabilities. The sedimentation characteristics and the association with light chain determinants and reduction by 2-mercaptoethanol lead us to believe that the cold reactive lymphocytotoxic factor is a 19S IgM antibody. The marked cytotoxicity for lymphocytes seen when the sucrose fractions associated with the 19S gradient fractions were tested at 37°C further support the hypothesis that lymphocytotoxic IgM antibodies may have *in vivo* functions.

ALA in malarious patients' serum may function as auto-regulatory 'feedback' antibodies. Since they react most strongly and in greatest amounts with B lymphocytes, they could function in modulating antibody production by the B lymphocytes. Hypothetically, autoreactive B cells may develop during malarial infection with the potential to synthesize antibodies against autologous cell subpopulations. Therefore it would be important to determine whether polyclonal B cell activation, which has been demonstrated in murine malaria (Rosenberg, 1978) can also be demonstrated in humans. Whether or not lymphocytotoxic activity in malarious serum results from activation of autoreactive B cells during the course of malaria infection can be of major theoretical and practical significance for future anti-malarial vaccine programs especially if an *in vivo* role is established for these lymphocytotoxic antibodies. Alternatively, a non-specific immunoregulatory role of lymphocytotoxic antibody would be consistent with the observation that serum cold reactive ALA have been reported in a variety of human disease states (Ozturk & Terasaki, 1979) as well as during pregnancy (Naito *et al.*, 1971) and following vaccination (Kreisler, Hirata & Terasaki, 1970). Thus, the cold reactive ALA may occur as a by-product of exposure of many different unrelated antigens.

An elevated level of red blood cell membranes fragments containing a variety of parasite antigens in the circulation of a malarious individual may, upon reaching a minimal level, be able to activate autoreactive B cell in a polyclonal fashion. Although there is little evidence at present to support this hypothesis one might speculate that the autolymphocytotoxins observed in pernicious anaemia patients (Goldberg, Cunningham & Terasaki, 1972) and malaria are due to the polyclonal activation of autoreactive B cells in response to altered RBC antigens. For example maturation or rupturing of parasitized erythrocytes may serve as an efficient method for presenting to the B cell either solubilized antigens from the RBC or altered RBC membranes which can stimulate production of ALA by autoreactive B cells. These ALA may cross-react with antigenic structures on lymphocyte subpopulations, i.e. the 'Ii' RBC antigen system. Similarly parasite derived antigens may induce the production of ALA which cross-react with receptors on subpopulations of MNC. In addition, anti-idiotypic antibody, reactive with determinants within or near a specific antigen binding site could function in this manner, since ALA have been shown to have anti-lymphocyte receptor activity (Nisonoff & Bangasser, 1975). The degree of avidity of the cytotoxic antibodies may vary since the IgM eluates are reactive at 37°C against B cells as previously observed in the study of Cicciarelli *et al.* (1980) on the identification of surface IgM as the target antigen of lymphocytotoxins. Furthermore, the purified IgM eluates demonstrated a higher level of ALA at 15°C than 37°C against highly enriched T cell populations. This data and the fact that highly

cytotoxic serum previously absorbed with enriched T cells or B cells continued to demonstrate ALA when tested at 15°C against the non-absorbing target cell type suggests the presence of distinct ALA with separate B and T cell specificities in patients sera. The ALA detected at 37°C in these experiments suggest that serum ALA are present in malarious individuals which could be capable of inter-reacting with peripheral blood B cells *in vivo* and which could be involved in modulating the humoral immune response to the malaria parasite.

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REFERENCES

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. lab. Invest.* **21,** 77.

CICCIARELLE, J.C., CHAI, D., TERASAKI, P.E., BARNETT, E.V. & SHIRAHAMA, S. (1980) Human IgM anti-IgM cytotoxin for B lymphocytes. *Tissue Antigens*, **15,** 275.

DEHORATIUS, R.J. & MESSNER, R.P. (1976) Lymphocytotoxins in acute and chronic hepatitis. *Clin. exp. Immunol.* **26,** 21.

GOLDBERG, L.S., CUNNINGHAM, J.E. & TERASAKI, P.I. (1972) Lymphocytotoxins and pernicious anemia. *Blood*, **39,** 862.

KREISLER, M.J., HIRATA, A.A. & TERASAKI, P.I. (1970) Cytotoxins in disease. III. Antibodies against lymphocytes produced by vaccination. *Transplantation*, **10,** 411.

KUNKEL, H.G. (1960) Macroglobulins and high molecular weight antibodies. In *The Plasma Proteins* (ed. by F.W. Putnam) Ch. 8. Academic Press, New York.

LIES, R.B., MESSNER, R.P. & WILLIAMS, R.C. (1973) Relative T cell specificity of lymphocytotoxins from patients with systemic lupus erythematosus. *Arthrit. Rheum.* **16,** 369.

MACDERMOTT, R.P. & STACEY, M.C. (1981) Further characterization of the human autologous mixed lymphocyte reaction (MLR). *J. Immunol.* **126,** 729.

MACDERMOTT, R.P., WELLS, R.A., ZOLYOMI, S., PAVANAND, K., PHISPHUMVIDHI, P., PERMPAMICH, B. & GILBREATH, M. (1980) Examination of peripheral blood mononuclear cells and sera from Thai adults naturally infected with malaria in assays of blastogenic responsiveness to mitogenic lectin and allogeneic cell surface antigen. *Infect. Immun.* **30,** 781.

NAITO, S., MICKEY, M.R., HIRATA, A. & TERASAKI, P.I. (1971) Anti-lymphocytotoxins following immunization by pregnancy, transplantation and disease. *Tissue Antigens*, **1,** 219.

NISONOFF, A. & BANGASSER, S.A. (1975) Immunological suppression of idiotypic specificities. *Transplant. Rev.* **27,** 100.

OZTURK, G. & TERASAKI, P.I. (1979) Non-HLA lymphocyte cytotoxins in various disease. *Tissue Antigens*, **14,** 52.

PARK, M.S., TERASAKI, P.I. & BERNOCO, D. (1977) Autoantibody against B lymphocytes. *Lancet*, **ii,** 465.

ROSENBERG, Y.J. (1978) Autoimmune and polyclonal B cell responses during murine malaria. *Nature*, **274,** 170.

TAYLOR, R.B., DUFFUS, W.P.H., RUFF, M.C. & DEPETRIS, S. (1971) Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by anti-immunoglobulin antibody. *Nature*, **233,** 225.

TOBIE, J.E., ABELE, D.C. & WOLFF, S.M. (1966) Serum immunoglobulin levels in human malaria and their relationship to antibody production. *J. Immunol.* **97,** 498.

WELLS, R.A., RAVANAND, K., ZOLYOMI, S., PERMPANICH, B. & MACDERMOTT, R.P. (1979) Loss of circulating T lymphocytes with normal levels of B and 'null' lymphocytes in Thai adults with malaria. *Clin. exp. Immunol.* **35,** 202.

WELLS, R.A., PAVANAND, K., ZOLYOMI, S., PERMPANICH, B. & MACDERMOTT, R.P. (1980) Anti-lymphocytotoxic antibodies in sera of Thai adults infected with *Plasmodium falciparum* or *Plasmodium vivax* malaria. *Clin. exp. Immunol.* **39,** 663.

WERNET, P., FOTINO, M., THOBURN, R., MOORE, A. & KUNKEL, H.G. (1972) Blockage of lymphocytes surface antigens and shedding phenomenon in systemic lupus erythematosus (SLE). (Abstract). *Arthrit. Rheum.* **16,** 137.

WINFIELD, J.B., WINCHESTER, R.J. & KUNKEL, H.G. (1975a) Association of cold-reactive antilymphocyte antibodies with lymphopenia in systemic lupus erythematosus. *Arthrit. Rheum.* **18,** 587.

WINFIELD, J.B., WINCHESTER, R.J., WERNET, P., FU, S.M. & KUNKEL, H.G. (1975b) Nature of cold-reactive antibodies to lymphocytes surface determinants in systemic lupus erythematosus. *Arthrit. Rheum.* **18,** 1.