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Comparison between autoantibodies in malaria and leprosy with lupus

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SUMMARY

Sera from 16 patients with falciparum malaria, 16 patients with vivax malaria and 31 patients with leprosy were tested for autoantibodies to intracellular proteins and nucleic acids. Precipitating antibodies to soluble protein extracts were not detected in any serum. Sera from malaria patients showed prominent immunofluorescence staining of the HEP2 nuclear membrane as well as frequent 75% (24/32) and intense Western blot reactivity. In contrast, only 20% and 36% of patients with leprosy had positive immunofluorescence or positive immunoblots respectively, and reactivity was weak in most cases. Neither the malaria nor leprosy sera contained autoantibodies with specificities similar to the characteristic lupus autoantibodies such as double stranded DNA (dsDNA), Ro/SSA, La/SSB, Sm, RNP and P proteins. Low levels of antibodies to single stranded (ssDNA) were however found in 11 (34%) malaria sera and in seven (23%) leprosy sera. Thirteen percent of patients with leprosy had anti-histone antibodies. These findings demonstrate considerable differences in the capacity of infectious agents to induce autoantibodies and also the infrequency with which autoantibodies characteristic of idiopathic systemic lupus erythematosus are induced.

Keywords malaria leprosy autoantibodies systemic lupus erythematosus

INTRODUCTION

Autoantibodies to intracellular proteins and nucleic acids are the hallmark of systemic lupus erythematosus (SLE) and related autoimmune diseases (Christian & Elkon, 1986). However, numerous studies have documented the presence of autoantibodies in infectious diseases such as malaria (Boonpucknavig & Ekapanyakuld, 1984; Adu *et al.*, 1982) and leprosy (Wager *et al.*, 1969; Shwe, 1972; Masala *et al.*, 1979; Bahr *et al.*, 1980). Antinuclear antibodies (ANA) have been reported to be present in approximately one-third of patients with *Plasmodium falciparum* infection (Phanuphak *et al.*, 1983) or leprosy (Bonomo *et al.*, 1965). Specifically, Boonpucknavig *et al.* (1983) have described the presence of antibodies to the Sm and RNP antigens in malaria, and McAdam *et al.* (1984) have reported the presence of anti-DNA antibodies in the sera of patients with leprosy. McAdam *et al.* have speculated that mycobacterial cell walls contain antigens which cross-react with the phosphate backbone of DNA leading to the production of anti-DNA antibodies in leprosy.

Since the specificities of autoantibodies produced in diseases where the infective agent and the pathogenesis are known could provide important clues to the mechanism of induction of

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autoantibodies in diseases such as SLE, we have partially characterized target antigens in malaria and leprosy and compared these to lupus. The results of this study indicate that autoantibodies are infrequently induced in leprosy infection and that although most patients with malaria produced antibodies that react with a heterogeneous variety of self proteins, these antigens are different from the major targets of lupus autoantibodies.

MATERIALS AND METHODS

Patients. Sera from 16 patients with falciparum malaria and 16 patients with vivax malaria were obtained from the Instituto de Saude, Belem, Brazil, and Instituto de Medicina Tropical, USP, Sao Paulo, Brazil. Sera from 31 patients with leprosy were obtained at the Instituto Arnaldo Vieira de Carvalho, Sao Paulo, Brazil. The disease classification of the leprosy patients was borderline (two), lepromatous (nine), tuberculoid (10), and indeterminate leprosy (10). The age of the leprosy patients ranged from 17 to 41 years, the mean age being 28 years. Sera were collected at the time of diagnosis of the disease, and before any treatment. Control sera were obtained from 20 healthy, age-matched Brazilians and 60 systemic lupus erythematosus (SLE) patients who met the revised criteria of the American Rheumatism Association (Tan *et al.*, 1982a). All SLE sera were known to contain antibodies to either Ro, La, Sm, RNP or ribosomal P antigens (Elkon & Jankowski, 1985). Reference sera with anti-histone antibodies from patients with idiopathic or drug-induced SLE were kindly provided by Dr J. Hardin (Yale University, CT, USA) and Dr P. Barland (Albert Einstein College of Medicine, NY, USA).

Autoantibody analysis. Antinuclear and anticytoplasmic antibodies were detected by indirect immunofluorescence (IIF) using a human epithelial cell line, Hep 2 (Antibodies Inc, CA) as substrate. Sera were diluted 1:50 in phosphate buffered saline (PBS) pH 7·4. Cryostat rat liver sections were also used as substrate. Counterimmunoelectrophoresis (CIE) was performed in 1·5% agarose in 0·05 M barbital buffer pH 8·2 (Kurata & Tan, 1976). Extract of rabbit thymus (RTE) and dog spleen (DSE) were employed as sources of antigens as described previously (Elkon & Culhane, 1984). One dimensional polyacrylamide gel electrophoresis (PAGE) was performed using the discontinuous buffer system of Laemmli in the presence of 0·1% sodium dodecyl sulphate (SDS) (Laemmli, 1970). Western blotting (Towbin, Staehelin & Gordon, 1979) was performed using dog liver extract (DLE) as a source of antigen as described previously (Elkon & Culhane, 1984).

Antibodies to dsDNA were detected using an enzyme-linked immunosorbent assay (ELISA). The assay was a minor modification of a previously described radioimmunossay for dsDNA (Mackworth-Young *et al.*, 1986). Plates were coated with poly-L-lysine, followed by calf thymus DNA 100 μg/ml overnight at 4°C. DNA was digested with S1-nuclease for 1 h at 37°C. Plates were then washed and blocked with 1% bovine serum and serially incubated with patient serum (diluted 1/50) and alkaline phosphatase anti-human γ-chain conjugate. After addition of the substrate, *p*-nitrophenylphosphate (1 mg/ml) in diethanolamine buffer, pH 9·8, the plates were incubated at room temperature. The reaction was stopped by adding 50 μl in each well and read on a Titertack multiscanner (Flow Laboratories) at 405 nm. Optical density values greater than 3 s.d. above the mean of 20 normals were considered positive. Anti-ssDNA antibodies were measured using a similar ELISA assay, except that ssDNA was prepared by boiling dsDNA in PBS pH 7·4 at 100°C for 15 min and chilling it in an ice bath. S1 nuclease treatment was omitted. An ELISA assay was developed to detect anti-histone antibodies. In brief, plates were coated with 50 μg/ml calf thymus histone (Sigma Chemical Co., St Louis, MO) in 0·01 N HCl in 4°C. The plates were then washed twice with PBS and blocked with 1% bovine serum albumin (BSA) in PBS. Wells were incubated for 3 h with a 1/50 dilution of patient serum in 1% BSA/PBS/0·05% Tween/10% normal goat serum (NGS) at room temperature. Anti-human IgG conjugate was diluted in the same buffer and added to the plate. The plate was washed and developed with substrate as described above.

Cells and subcellular fractionation. HeLa cells were cultured in suspension using Joklik's modified minimal essential medium supplemented with 5% bovine calf serum (BCS), 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Nuclear and cytoplasmic extracts from HeLa cells were made by Dounce homogenization of whole cells in hypotonic buffer (10 mM KCl,

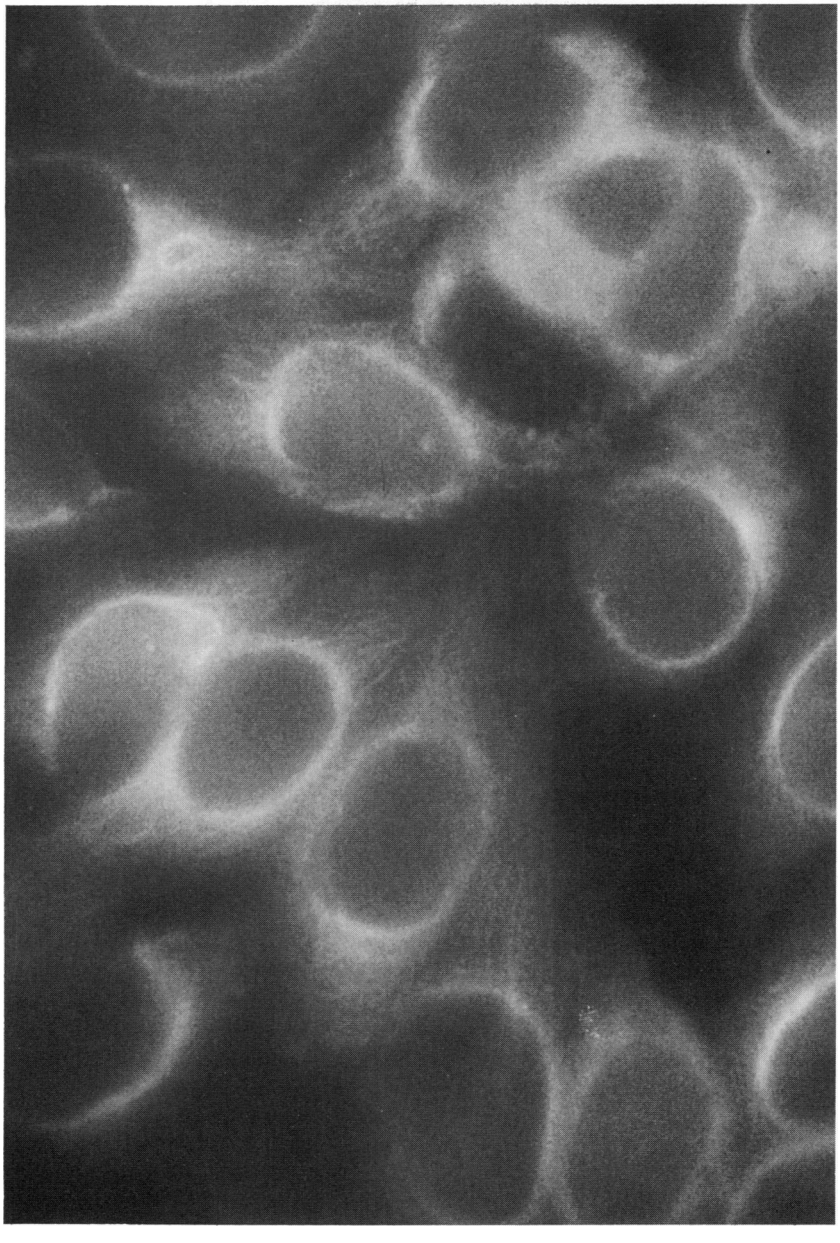

Fig. 1. Characteristic immunofluorescence pattern from a malaria serum showing strong reactivity with the nuclear membrane and filamentous cytoplasmic staining. Hep-2 cells were used as substrate (original magnification × 900).

1·5 mM $MgCl_2$, 10 mM Tris HCl, pH 7·8) and Dounce homogenization of washed nuclei in a hypertonic buffer (20 mM Tris HCl, pH 7·8, 20% glycerol, 0·4 M NaCl, 1·5 mM $MgCl_2$, 0·1 mM PMSF, 0·5 mM DTT) as described previously (Elkon & Jankowski, 1985). Whole cell extracts were made by washing cells and resuspending the cells in Laemmli sample buffer containing 2% SDS.

Table 1. Frequency of autoantibodies in leprosy and malaria patients

	Patients	CIE	IEF				WB
			Nuclear speckled	Nuclear membrane	Cytoplasmic speckled	Cytoplasmic filamentous	
Malaria falciparum	16	0	5 (31%)	12 (75%)	6 (38%)	4 (25%)	14 (88%)
vivax	16	0	4 (25%)	14 (88%)	8 (50%)	3 (19%)	10 (63%)
Leprosy lepromatous	9	0	0	0	0	0	3 (33%)
borderline	2	0	1	0	0	0	1
tuberculoid	10	0	4 (40%)	0	3 (30%)	1 (10%)	4 (40%)
indeterminate	10	0	0	0	1 (10%)	0	3 (30%)

CIE, counterimmunoelectrophoresis.
IEF, indirect immunofluorescence.
WB, Western blotting.

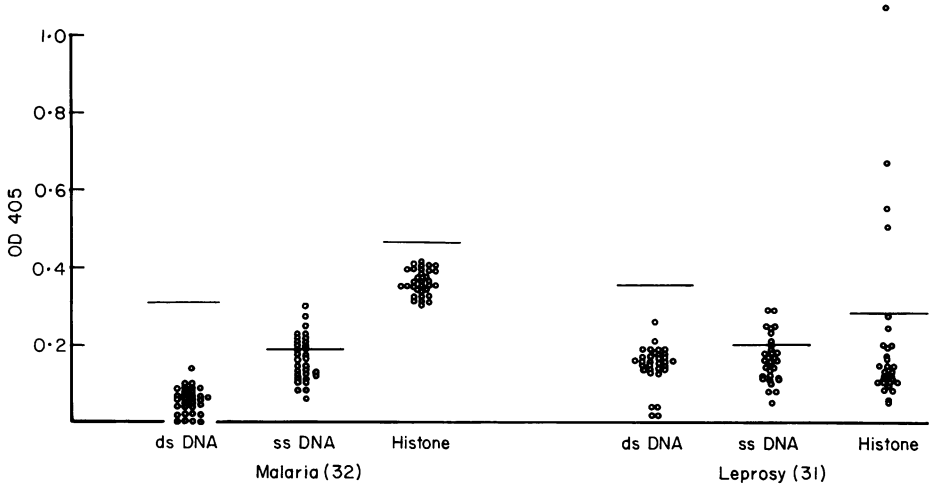

Fig. 2. Anti-dsDNA, anti-ssDNA and anti-histone activity in 32 malaria patients and 31 leprosy patients as measured by ELISA. Horizontal lines indicate 3 standard deviations above the means of 20 normal Brazilian controls.

RESULTS

Detection of autoantibodies by counterimmunoelectrophoresis and indirect immunofluorescence. All malaria and leprosy sera were negative for precipitins to saline soluble proteins and ribonucleoproteins as determined by CIE. Twenty-six (81%) of the malaria sera reacted with the Hep 2 nuclear membrane (Fig. 1) and nine (28%) patients produced a positive nuclear speckled pattern. The predominant cytoplasmic staining, present in 14 (44%) patients, was speckled but filamentous staining was seen in seven (22%) patients. As shown in Table 1, similar frequencies of antibodies were seen in patients with vivax and falciparum malaria. Twelve of the malaria sera which produced strong nuclear membrane fluorescence (including three sera with additional speckled staining) were analysed on rat liver sections. None of these sera stained the rat liver cell membranes although the speckled nuclear fluorescence was reproduced in the three positive sera. In

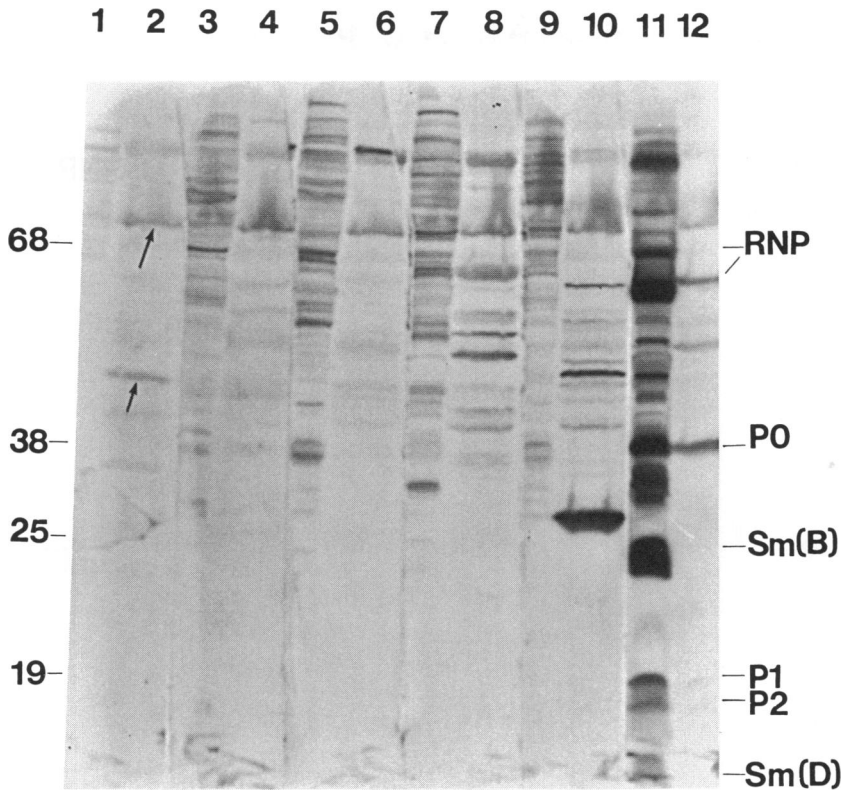

Fig. 3. One dimensional immunoblot showing the major antigens recognized by 4 different malaria patients. The source of antigens were HeLa nuclear extracts (lanes 1, 3, 5, 7, 9 and 11) and dog liver (lanes 2, 4, 6, 8, 10 and 12). Lanes 1 and 2 were probed with normal serum, lanes 3, 4, 5, 6, 7, 8, 9 and 10 with 4 different vivax malaria sera, lanes 11 and 12 with lupus serum containing anti-Sm, RNP and P antibodies (shown on the right). Molecular weights in kD are shown on the left.

contrast, of the 31 leprosy sera tested, only six (20%) produced positive immunofluorescence. Furthermore, the speckled nuclear fluorescence and the cytoplasmic staining was weak in all positive patients. Neither nuclear nor cytoplasmic staining was observed when sera of the nine patients with lepromatous leprosy were tested (Table 1).

Detection of autoantibodies by ELISA. The frequencies of autoantibodies to dsDNA, ssDNA and histone in malaria and leprosy patients are shown in Fig. 2. Antibodies to dsDNA were not detected. Low levels of antibodies to single stranded ssDNA were however found in 11 (34%) malaria sera and seven (23%) leprosy sera. Malaria sera were uniformly negative for anti-histone antibodies while four (13%) of leprosy sera had moderate to high titres of anti-histone antibodies.

Characterization of antigens recognized by malaria and leprosy sera. Seventy-five percent (24/32) of the malaria sera were positive by Western blot analysis using HeLa nuclei or dog liver extract as an antigen source (Table 1). Nine of these sera produced speckled nuclear fluorescence staining on Hep 2 cells. Protein antigens were not detected by Western blotting in seven immunofluorescence positive patients, presumably reflecting antibody reactivity with ribonucleoprotein (RNP) complexes or protein conformations denatured by SDS. Considerable heterogeneity in reactivity was evident on immunoblots (Fig. 3). The proteins recognized by malaria sera were however different from those targeted by lupus sera (Fig. 3) with known specificities for Ro, La, Sm, RNP, P and other antigens of uncertain nature (Elkon & Jankowisk, 1985). Eighty-eight percent of the patients with falciparum malaria tested by Western blotting had autoantibodies reactive against

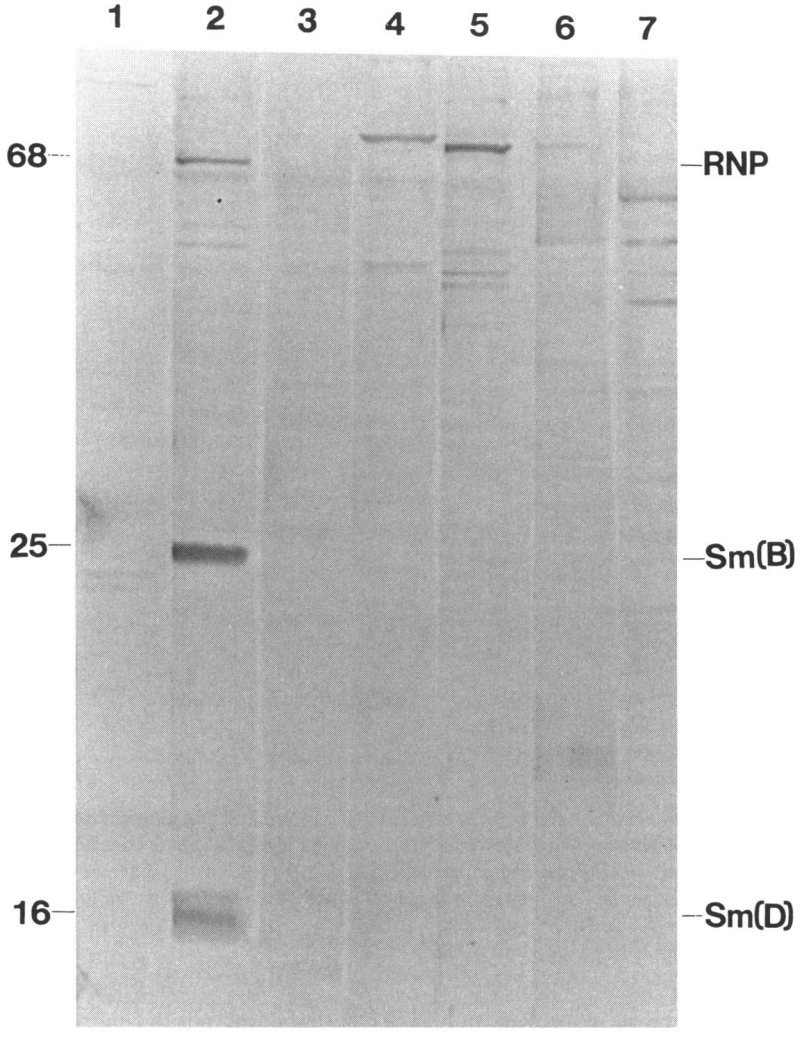

Fig. 4. Immunoblot analysis of a HeLa whole cell extract probed with sera from patients with leprosy. Lane 1 was probed with normal serum, lane 2 with lupus serum containing anti-Sm and RNP antibodies (shown on the right) and lanes 4, 5, 6, 7 and 8 with five different immunofluorescence positive leprosy sera. Molecular weights in kD are shown on the left. Lanes 4, 5 and 7 were scored as positive and lanes 3 and 6 as negative.

HeLa cell and/or dog liver extracts, whereas 63% of the patients with vivax malaria were positive using the same extracts (Table 1). Most of the positive sera reacted with different proteins in each extract, and more than one protein was recognized by the majority of patients (Fig. 3). No consistent pattern was therefore observed. Weak reactivity with proteins of approximate molecular weights 74 kD and 45 kD were observed when normal sera were used to probe the dog liver extract (lane 2, Fig. 3). Since all sera showed binding to these proteins, this reactivity was interpreted as non-specific and was not scored as positive.

Of the 31 leprosy sera tested by Western blotting using HeLa whole cell extract as an antigen source, 11 (36%) were positive (Table 1). Three of these sera had nuclear and cytoplasmic staining when tested on Hep2 cells. In three immunofluorescence positive patients, antigens were not detected by Western blot analysis, again presumably reflecting antibody reactivity with RNP complexes or protein conformations denatured by SDS. In contrast to the heterogeneous reactivity

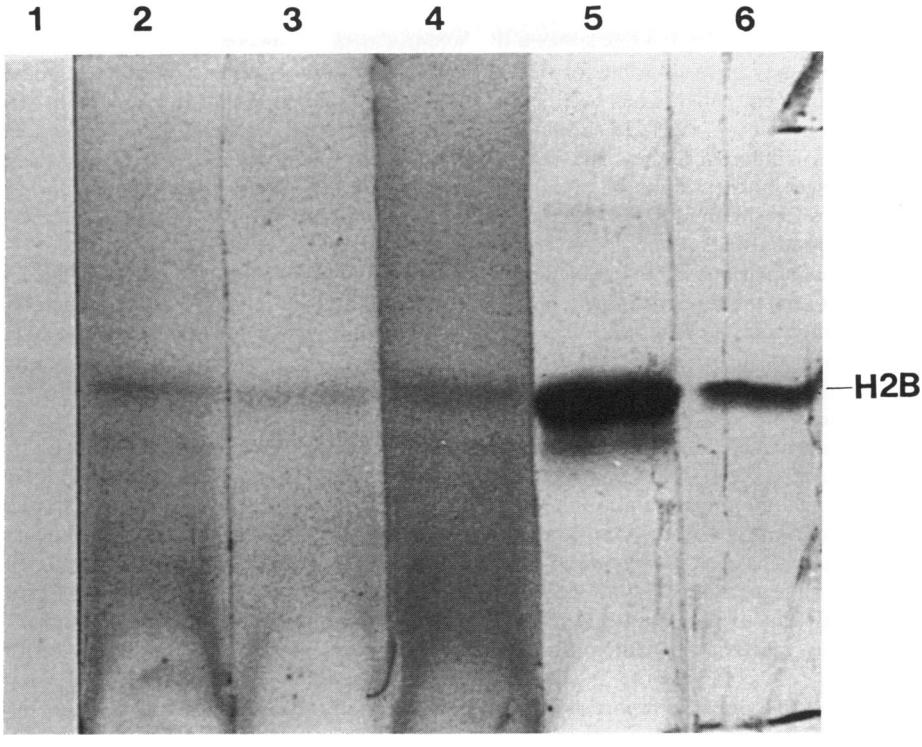

Fig. 5. Immunoblot analysis of calf thymus histones. Lane 1 was probed with normal serum, lanes 2, 3 and 4 with three different leprosy sera which were positive for anti-histone on ELISA. Lanes 5 and 6 were probed with SLE sera known to contain anti-histone H2B antibodies (shown on the right).

observed in malaria patients, leprosy sera showed limited binding to HeLa cell proteins. Figure 4 shows the immunoblot reactivity of five selected immunofluorescence positive leprosy sera. Binding was for most patients weak and common antigen specificities were rarely observed. None of these proteins were of similar molecular weight to the lupus autoantigens. The presence of anti-histone antibodies in leprosy sera was confirmed by Western blot analysis as shown in Fig. 5. Although binding was predominantly to histone H2B, antibody reactivity was considerably weaker than that of the lupus control sera (lanes 5 and 6).

DISCUSSION

The major findings in this study are that patients with both acute and chronic malaria infections show prominent autoantibody reactivity against a variety of intracellular proteins by Western blot analysis. In contrast, patients with a chronic mycobacterial infection produced autoantibodies less frequently. In neither case were autoantibodies characteristic of SLE observed. Although only IgG autoantibodies were studied in this report, it is possible that autoantibodies of other immunoglobulin classes may be present.

A high frequency of ANA in falciparum malaria has previously been reported (Adu *et al.*, 1982). The finding of speckled staining in 28% of the patients with malaria in the present study is in agreement with previous studies (Phanuphak *et al.*, 1983). However, using Hep 2 cells, both vivax and falciparum malaria produced prominent nuclear membrane staining. This difference in immunofluorescence patterns is most likely related to the substrate used since we could not detect nuclear membrane immunofluorescence on rat liver sections. A lower frequency of ANA was

observed in leprosy. In contrast to previous reports (Saha, Dutta & Mittal, 1975; Kreisler *et al.*, 1975), we did not find a more prevalent positive ANA in the lepromatous group. It is possible that genetic factors may be responsible for this discrepancy, since the frequency of autoantibodies in leprosy among Thai patients has been shown to be very different from Caucasian and African patients (Petchalai *et al.*, 1973). In addition, considerable variation in the dilution of patient serum has been used in different studies. The frequency of positive immunofluorescence was, for the most part, paralleled by the results of immunoblotting experiments. The majority of malaria sera produced positive immunoblots with reactivity against several proteins whereas leprosy sera generally showed a weaker and less heterogeneous reactivity.

Possible explanations for the induction of autoantibodies in infectious diseases include cross-reactivity between the foreign antigens and host proteins, modification of host proteins and direct infection or modulation of the cells of the immune system. Falciparum malaria is subacute or acute whereas vivax and leprosy are chronic diseases so that duration of exposure is unlikely to be a major factor. Both leprosy and malaria directly or indirectly effect cells of the immune system. In leprosy, the *Mycobacterium* resides within macrophages and malaria infections have been reported to produce mitogens that activate B cells polyclonally (Greenwood, 1974), possibly through a T cell factor (Rosenberg, 1978). Additional factors that may explain the high frequency of autoantibodies in malaria are the continued catabolism of host cells (erythrocytes) and perhaps more importantly, antigenic variation on the surface of the parasite (Hommel *et al.*, 1983). It has been shown that several immunogens of *P. falciparum* contain repeats with related sequences (Anders *et al.*, 1986). Anders *et al.* have speculated that these repeats play an important role in 'baffling' the host immune response and lead to an increase in the number of somatic mutants with autoreactivity. The heterogeneous reactivity of autoantibodies detected in this report is compatible with this hypothesis.

In contrast to a previous report of anti-Sm and RNP antibodies in the serum of patients with malaria, we found no evidence for these antibodies by counterimmunoelectrophoresis nor by immunoblotting in any of the 32 patients with malaria tested. Since neither of these diagnostic procedures were followed by Boonpucknavig & Ekapanyakuld (1984) these authors may have detected other autoantibodies. Anti-dsDNA antibodies are highly specific for SLE (Tan, 1982b) whereas anti-ssDNA antibodies are frequently demonstrated in other diseases (Koffler *et al.*, 1969). MacAdam *et al.* (1984) have found anti-DNA antibodies in leprosy sera and have suggested that cross-reactions with mycobacteria may induce autoantibodies. Such cross-reactions may have occurred with ssDNA since our findings and those of Rea *et al.* (1976) and Miller *et al.* (1987) indicate that autoantibodies to dsDNA are not present in leprosy sera. Similarly, the low levels of antibodies to ssDNA detected in eleven malaria sera could arise from a cross-reaction between ssDNA and phospholipids produced by the parasite (Ribeiro *et al.*, 1984). A minority of leprosy patients produced anti-histone antibodies. The significance of this finding remains to be determined since antibodies to histone have been described in high frequency in drug induced lupus and in lower frequency in spontaneous lupus (Tan, 1982b).

The inability to detect antibodies to the frequent targets of lupus autoantibodies (dsDNA, Sm, RNP, Ro, La and P) in malaria, leprosy, infective endocarditis (Elkon *et al.*, 1983) or in a variety of ANA positive neoplastic diseases (Bonfa, Bystryn & Elkon, in press) suggest that the inducing stimuli or cellular defects in SLE are highly specific.

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REFERENCES

ADU, D., WILLIAMS, D.G., QUAKYI, I.A., VOLLER, A., ANIM-ADDO, Y., BRUCE-TAGOE, A.A., JOHNSON, G.D. & HOLBOROW, E.J. (1982) Anti-ssDNA and antinuclear antibodies in human malaria. *Clin. exp. Immunol.* **49**, 310.

ANDERS, R.F., SHI, P.T., SCANLON, D.B., LEACH, S.J., COPPEL, R.L., BROWN, G.V., STAHL, H.D. & KEMP, D.J. (1986) Antigenic repeat structures in proteins of *Plasmodium falciparum*. Synthetic peptides as antigen. Ciba Foundation Symposium **119**, p. 164. Wiley, Chichester.

BAHR, G.M., ROOK, G.A.W., MORENO, E., LYDYARD,

P.M., MODABBER, F.Z. & STANFORD, J.L. (1980) Use of the ELISA to screen for anti-thymocyte and anti-β_2-microglobulin antibodies in leprosy and SLE. *Immunology* **41,** 865.

BONFA, E., BYSTRYN, J.-C. & ELKON, K.B. (1987) Detection of IgG antibodies in melanoma sera reactive with intracellular proteins. *J. Inv. Dermatol*, in press.

BONOMO, L., TURSI, A., TRIMIGLIOZZI, G. & DAMMACCO, F. (1965) L. E. cells and antinuclear factors in leprosy. *Brit. med. J.* **ii,** 689.

BOONPUCKNAVIG, S. & EKAPANYAKULD, G. (1984) Autoantibodies in sera of Thai patients with *Plasmodium falciparum* infection. *Clin. exp. Immunol.* **58,** 77.

CHRISTIAN, C.L. & ELKON, K.B. (1986) Autoantibodies to intracellular proteins, clinical and biological implications. *Am. J. Med.* **80,** 53.

ELKON, K.B., INMAN, R.D., CULHANE, L. & CHRISTIAN, C.L. (1983) Induction of polymeric IgA rheumatoid factors in infective endocarditis. *Am. J. Med.* **75,** 785.

ELKON, K.B. & CULHANE, L. (1984) Partial immunochemical characterization of the Ro and La proteins using antibodies from patients with sicca syndrome and lupus erythematosus. *J. Immunol.* **132,** 2350.

ELKON, K.B. & JANKOWSKI, P.W. (1985) Fine specificities of autoantibodies directed against Ro, La, Sm, RNP and Jo-1 proteins defined by two-dimensional electrophoresis and immunoblotting. *J. Immunol.* **134,** 3819.

GREENWOOD, B.M. (1974) Possible role of a B-cell mitogen in hypergammablobulinaemia in malaria and trypanosomiasis. *Lancet* **i,** 435.

HOMMEL, M., DAVID, P.H. & OLIGINO, L.D. (1983) Surface alterations of erythrocytes in *Plasmodium falciparum* malaria. Antigenic variation, antigenic diversity, and the role of the spleen. *J. exp. Med.* **157,** 1137.

KOFFLER, D., CARR, R.I., AGNELLO, V., FIEZI, T. & KUNKEL, H.G. (1969) Antibodies to polynucleotides: distribution in human serums. *Science* **166,** 1648.

KREISLER, J.M., ARNAIZ, A., PEREZ, B. & BOOTELLO, A. (1975) Lymphototoxins in leprosy. *J. Int. Lepr.* **43,** 91.

KURATA, N. & TAN, E.M. (1976) Identification of antibodies to nuclear acidic antigens by counterimmunoelectrophoresis. *Arthritis Rheum.* **19,** 574.

LAEMMLI, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680.

MASALA, C., AMENDOLEA, M.A., NUTI, M., RICCARDUCCI, R., TARABINI, C.G.L. & TARABINI, C.G. (1979) Autoantibodies in leprosy. *Int. J. Lepr.* **47,** 2.

MACADAM, K.P.W.J., MUDD, D., SHOENFELD, Y. *et al.* (1984) Autoantibodies to DNA in leprosy: antigenic similarities between DNA and mycobacterial phospholipids defined by human monoclonal antibodies. *Int. J. Lepr.* (suppl) **52,** 697.

MACKWORTH-YOUNG, C.G., CHAN, J.K.H., BUNN, C.C., HUGHES, G.R.V. & GHARAVI, A.E. (1986) Complement fixation by anti-DNA antibodies in SLE: measurement by radioimmunoassay and relationship to disease activity. *Ann Rheum Dis* **45,** 314.

MILLER, R.A., WENER, M.H., HARNISCH, J.P. & GILLILAND, B.C. (1987) The limited spectrum of anti-nuclear antibodies in leprosy. *J. Rheumatol.* (in press).

PHANUPHAK, P., TIRAWATNPONG, S., HANVANICH, M., PANMUONG, W., MOOLLAOR, P., VEJJAJIVA, S., SITPRIJA, V., INTARAPRASERT, R. & PHANTHUMKOSOL, D. (1983) Autoantibodies in falciparum malaria: a sequential study in 1983 Thai patients. *Clin. exp. Immunol.* **53,** 627.

PETCHCLAI, B., CHUTHANONDH, R., RUNGRUONG, S. & RAMASOOTA, T. (1973) Autoantibodies in leprosy among Thai patients. *Lancet* **1,** 1481.

REA, T.H., QUISMORIO, F.P., HARDING, B., NIES, K.N., DISAIA, P.J., LEVAN, N.E. & FRIOU, G.J. (1976) Immunologic responses in patients with lepromatous leprosy. *Arch. Dermatol.* **112,** 791.

RIBEIRO, C.T.D., ROQUEFEUIL, S., DRUILHE, P., MONJOUR, L., HOMBERG, J.C. & GENTILINI, M. (1984) Abnormal anti-single stranded ssDNA activity in sera from *Plasmodium falciparum* infected individuals. *Trans. R. Soc. trop. Med. Hyg.* **77,** 185.

ROSENBERG, Y.J. (1978) Autoimmune and polyclonal B-cell responses during murine malaria. *Nature* **193,** 170.

SAHA, K., DUTTA, R.N. & MITTAL, M.M. (1975) Immunologic aspects of leprosy as related to leucocytic isoantibodies and platelet aggregating factors. *Int. J. Lepr.* **43,** 239.

SHWE, T. (1972) Clinical significance of autoimmune antibodies in leprosy. *Trans. R. Soc. trop. Med. Hyg.* **66,** 749.

TAN, E.M., COHEN, A.S., FRIES, J., MASI, A.T., MCSHANE, D., ROTHFIELD, N.F., SCHAKKER, J., TALAL, N. & WINCHESTER, R. (1982a) Criteria for classification of systemic lupus erythematosus. *Arthritis Rheum* **27,** 530.

TAN, E.M. (1982b) Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. *Adv Immunol.* **33,** 167.

TOWBIN, H., STAEHELIN, T. & GORDON J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. natn Acad. Sci. USA.* **76,** 4350.

WAGER, D. (1969) Immunological aspects of leprosy with special reference to autoimmune diseases. *Bull. WHO* **41,** 793.