Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Suppl. I Vol. 83, November, 1988/Page 633

POLYCLONAL B-CELL ACTIVATION (PBA) CH-87-24

WHAT HAVE WE LEARNED FROM THE STUDY OF MALARIA?

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THE DIVERSITY OF IMMUNE RESPONSE IN MALARIA

Human and experimental rodent malaria are accompained by a marked increase in the serum immunoglobulin (Ig) levels (Abele et al., 1965; Poels & Van Niekerk, 1977). However absorbtion studies have shown that only a minor part of the produced Igs is especific to plasmodial antigens (Ags) (Curtain, et al., 1964; Freeman et al.,1970). The major part is of unknown specificity and may contain antibodies (Ab) directed against several Ags; hetero-Ags such as red blood cells (RBC) from sheep, horse, rabbit, guinea pig and rat (Adenyi-Jones, 1967; Kano, McGregor & Milgron, 1968; Greenwood, 1970; Houba, Page-Faulk & Matola, 1974; Freeman & Parish, 1978; Rosenberg, 1978; Weinbaum et al., 1978) and Auto-Ags such as nuclear Ags (Kreier & Dilley, 1969; Greenwood, Herrick & Holborow, 1970; Voller, O'Neill & Humphrey, 1972; Quakyi et al., 1979 Poels et al., 1980; Adu et al., 1982; Daniel Ribeiro et al., 1983a, 1984b; Zouali, Druilhe & Eyquem, 1986) immunoglobulins (Houba & Allison, 1966; Shaper et al., 1968; Greenwood, Muller & Valkenburg, 1971; Quakyi et al., 1979) smooth muscle Ags (Poels, Van Niekerk & Jerusalem, 1978; Poels et al., 1980; Quakyi et al., 1979; Daniel Ribeiro,1983), RBC Ags (Rosenberg, 1978; Le Francois et al.,1981) and even organ specific Ags (Shaper et al., 1968).

Such a diversity of the immune response lead Greenwood (1974) to postulate the existence of a parasite derived B-cell mitogen to explain the hypergammaglobulinaemia characteristic of the disease.

PARASITE MITOGENS AND PBA, A CAUSE-EFFECT RELATIONSHIP?

Studying the proliferative response of peripheral blood lymphocytes (PBL) to phytohaemagglutinin (PHA) in malarious children, Osunkoya, Williams & Reddy (1972)

observed a spontaneous lymphocytic transformation in non stimulated cultures. A few years later, Wyler & Oppenheim (1974) showed that a **Plasmodium falciparum** RBC lysate could stimulate PBL from normal non infected individuals. Similar results were then reported by Greenwood & Vick (1975) demonstrating the mitogenic properties of supernatants of short term **in vitro** culture of **Plasmodium falciparum** and of lysates of infected RBC. Sera from infected individuals however was only found to possess mitogenic factors when murine lymphocytes were used as target cells (Strickland, 1978).

Using the model of experimental rodent malaria Jayawardena et al., (1975) and Freeman & Parish (1978) recorded respectively a T-cell activation and a polyclonal B-cell stimulation among infected animals. Freeman & Parish considered the possibility that the parasite mitogen is a T-cell mitogen and that the PBA is in fact a T-dependent effect. A first evidence for this was brought by the work of Rosenberg (1978) who noted that the PBA recorded in the course of **P. yoelii** (17 XL) infection was absent in nude mice. Weinbaum, Evans & Tigelaar (1976) and Freeman & Parish (1978) showed thereafter that lysates of **P. yoelii** or of **P. berghei** infected RBC could stimulate lymphocytes from normal mice to synthesize Ab. Weinbaum and coworkers have also shown that the proliferative response was decreased when responding lymphocytes were pre-treated with anti-θ serum and that only 25% of blasts generated by parasite extract stimulation were surface Ig carrying cells (compared to 90% of blasts induced by the B-cell mitogen LPS). These findings suggest the involvement of T-cell in the process of cell activation.

In 1979, Greenwood, Oduloju & Platts-Mills observed that both T- and B- lymphocytes were involved in the lymphocyte proliferation induced by **P. falciparum** extracts. In the same year, however, Wyler, Herrod & Weinbaum (1979) clearly showed that the parasite mitogen is in fact a T-cell dependent mitogen and that mixed populations containing 99% of B-cell and 1% of T-cell proliferate while pure B-lymphocytes preparations do not. Like Freeman & Parish (1978) these authors considered the possibility that B-lymphocytes are activated secundarily to a T-cell activation.

These reports are complicated by the fact that the parasite extracts simultaneously exhibited antigenic and mitogenic properties and in some studies the distinction between these activities was unclear since normal control individuals were living in endemic areas under chemoprophylatic treatment (Greenwood & Vick, 1975; Greenwood, Oduloju & Platts-Mills, 1979). In some other cases the parasite preparations might have retained some heterogeneous material derived fom monkey erythrocytes or from the foetal calf serum used in maintainig the parasites (Greenwood & Vick, 1975; Wyler, & Oppenheim, 1974). This problem was definitively solved by a well designed study by

Ballet et al.(1981) who used for mantaining **P. falciparum**, human foetal serum and, as normal controls, europeans that had never been in malaria endemic areas. These authors have also described a T-cell especific mitogen in the supernatants of continuous **P. falciparum in vitro** culture.

Finally, in the same year, Bird et al. (1981), showed that polyclonal T-cell activation induced by T-cell mitogens can secondarily induce a polyclonal B-cell response and Teodorescu (1981) noted the presence of a substance endowed with PBA properties in the sera of animals injected with T-cell mitogens.

More recently, Langhorne, Kim & Asofsky (1985) and Falanga et al. (1987) studied the isotypic pattern of polyclonal B-cell responses in **Plasmodium chabaudi** (primary infection and immune protected mice) and **Plasmodium yoelii** 17XNL and showed that different patterns of PBA were observed according to the species and to the immune status of animals studied.

PBA AND INDUCTION OF AUTOANTIBODY PRODUCTION: HOW DOES IT OCCUR?

Malaria provides therefore a model of study in which the host remains immunologically normal, tolerant to auto-Ags, until its immune system is exposed to parasite derived substances endowed with powerful properties of PBA. We can thus suppose that the mitogenic effects of **Plasmodium** are in the origin of auto-Ab production observed during the course of infection.

In fact, the induction of auto-Ab synthesis by PBA is now a well documented phenomenon both **in vivo** (Fournie, Lambert & Miescher, 1974; Cunningham, 1976; Izui et al., 1977; Nakashima et al., 1977; Primi et al., 1977a) and **in vitro** (Hammarstrom et al., 1976; Primi et al., 1977b; Beall & Krugger, 1980). It remains however to be determined if PBA can stimulate autoreactive B-cells (ARC) by delivering only one stimulatory signal, independently of the presence of the Ag, as previously proposed in the "one signal" theory for B-lymphocyte activation (Coutinho & Moller, 1974). We can also wonder if PBA can only act as a source of a second signal to cells that have already received a first (specific) signal delivered by Ig receptors in contact with specific Ag (Bretscher & Cohn, 1970; Cohn & Blomberg, 1975). Some reports seem to indicate that PBA can directly activate ARC in a non specific way (Hammarstrom et al., 1976; Izui et al., 1977; Primi et al., 1977a b); other results suggest that the autoimmune responses induced by mitogens are not a direct consequence of a polyclonal stimulation of ARC but need the presence of the Ag in the **in vivo** or **in vitro** system (Fournie, Lambert & Miescher, 1974; Esquivel, Rose & Kong, 1977; Nakashima et al., 1977; Daniel-Ribeiro et al., 1982).

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In the last years we have been involved in experimental works the results of which seem to support the "two signal" view of B-cell activation. The production of auto-Ab during malaria was chosen as model of study since in that case (rather than in the situation of immune response to heterologous Ags) the presence of parasite mitogen (an exogenous PBA) can constitute a substitute for helper T-cells (source of physiological PBA) and furnish a stimulatory (PBA) signal that would be otherwise absent (since helper T-cells are inoperant in the case of Auto-Ags). In that way a "yes or no phenomenon"(in terms of Ab production) will be analysed rather than the subjective situation of assessing the potentiation of pre-existing helper effects by parasite PBA.

One of the first indications of a "two signal" mechanism of activation of ARC by PBA during the infection came with the analysis of the specificities involved in the autoimmune phenomenon associated to malaria.

In 1983,sera from 182 individuals living in a malaria endemic area (Donse village) in the Upper-volta (Bourkina Faso) were tested, by indirect immunofluorescence and passive haemaglutination, for the presence of 13 different anti-tissular auto-Abs (Daniel Ribeiro et al., 1983a). Antinuclear Abs, of a specific (speckled) pattern of fluorescence, was found in 68% of the invividuals studied. These Abs were not related to the age or sex of individuals but clearly associated with high levels of malaria Abs and of serum IgM. Smooth muscle Abs (SMA), heart, gastric parietal cell and thyroglobulin Abs were found at normal frequencies. Other antitissue auto-Abs were not observed. It was concluded that this selective increase in the frequency of one auto-Ab (and not of others) could not result from a non specific PBA and provides an indirect evidence against the universal triggering of B-cells that one would expect two occur if the auto-Ab formation were entirely dependent on the stimulatory effects of parasite mitogens.

The normal frequency of anti-thyroglobulin antibodies (Daniel Ribeiro et al., 1984a) suggested also that the auto-Ab observed could not be explained only by PBA since B-lymphocytes reacting with the thyroglobulin autoantigen exist in healthy people (Bankhurst, Torrigiani & Allison, 1973; Roberts, Whittingham & Mackay, 1973) and we should have expected activation of these ARC by malaria parasite mitogen. In a model of LPS induced thyroiditis (Esquivel, Rose & Kong, 1977), the autoimmune response to the thyroglobulin,a partially sequestered auto-Ag,could only be observed if the auto-Ag itself was simultaneously administred. On the basis of these data we proposed that the formation of auto-Abs during malaria is a "two signal" effect (Daniel Ribeiro et al., 1983a) rather than the "one signal" phenomenon proposed by Coutinho & Moller (1974). This dependence on the auto-Ag for the ARC activation by

parasite mitogens could explain, at least partially, the differences in the pattern of autoimmune response observed during the course of parasitic infections endowed with PBA properties, e.g. anti-DNA Abs in African Trypanosomiasis (Lindsley, Kysela & Steinberg, 1974; Daniel Ribeiro et al.,1983 b), anti-neurone Abs in Chagas disease (Khoury et al., 1979) and speckled anti-nuclear Abs in malaria infection (Greenwood, Herrick & Holborow, 1970; Voller, O'Neill & Humphrey, 1972; Quakyi et al., 1979; Daniel Ribeiro et al., 1983 a). In fact, in each parasitic infection, the parasite Ags cross-reactive with host Ags and/or the host target organs (and consequently the nature of the released auto-Ags) would be different.

To seek further support for this hypothesis, advantage was taken of the existence in man of lymphocytes autoreactive with DNA (Bankhurst & Williams, 1975) and of the fact that DNA is a non-organ specific auto-Ag that could be released in immunogenic amounts by nucleated host cells, parasitized or not, or by **Plasmodium** itself, in the course of infection. Sera from 32 subjects with **Plasmodium falciparum** parasitaemia were screened for the presence of Abs to native-double stranded (ds) DNA and to heat-denaturated-single stranded (ss) DNA by a Farr DNA binding radioimmunoassay (Daniel Ribeiro et al., 1984 b). Anti-dsDNA Abs were also studied by indirect immunofluorescence using **Crithidiae luciliae** and rat liver sections as substrates. Immunoglobulin (G, A, M) levels and Plasmodial Abs (PA) titres were concomitantly evaluated.

The anti-ssDNA activity was found to be higher in malarious individuals with high levels of IgM. This activity was higher during the acute stage of infection than after recovery. A positive and significant relationship was found between the anti-ssDNA activity and the IgM level but not with IgG, IgA or PA titres. Speckled anti-nuclear Abs (ANA) were also observed in 43.8% of the individuals and the mean anti-ssDNA activity was higher in these ANA positive patients. Conversely anti-dsDNA Abs could not be detected by any of the tests performed. This preferential production anti-ssDNA Ab and not of anti-dsDNA Ab was also interpreted as additional evidence that the auto-Abs observed in malaria infection are not consequence of a generalized and non-specific PBA. Indeed dsDNA reactive B-lymphocytes could constitute target cells for the stimulatory effects of **P. falciparum** mitogens and one would expect to observe synthesis of anti-dsDNA Abs if the activation of ARC by mitogens were a non specific phenomenon. Once again the activation of ARC by PBA during malaria was interpreted as a result of a specific phenomenon depending on the presence of the corresponding auto-Ag (Daniel Ribeiro et al., 1984b). To explain the source of ssDNA in this "two signal" way of B-cell activation two, non exclusive, mechanisms were proposed. Firstly that the dsDNA released in the circulation by parasite or by nucleated host cells could be catabolised rapidly (as it is in mice, Chesud, Steinberg & Talal, 1972)

and could remain available, in its denaturated form, to activate, in the presence of the parasite mitogen, the corresponding ARC. The other possibility is that the antigenic stimulatory signal could arise from cross-reactions that exist between ssDNA and phospholipids (Shoenfeld et al., 1983) since it is now well documented that abnormally increased levels of (parasite produced) phospholipids are observed in malaria infected individuals (Vial et al., 1982).

The dependence of auto-Ag for ARC activation by PBA during malaria was finally confirmed in a experimental model of **P.yoelii** (17 XL) rodent malaria. Although this strain of **Plasmodium** induces a PBA status in parasitized mice neither infected animals nor mice injected with the B-cell mitogen LPS presented Abs against the organ specific partially sequestered auto-Ag thyroglobulin (Tg). However these Abs could be induced in both groups of mice if the auto-Ag Tg was injected simultaneously with the infection or LPS injection (Daniel Ribeiro et al.,1982).

Taken together these results strongly indicate that the auto-Ab formation during malaria infection is a "two signal" specific effect dependent on the presence of the auto-Ag rather than a consequence of a universal triggering of all ARC by parasite mitogens.

THE COMPOSITION OF PBA AND HETERO-AB PRODUTION DURING MALARIA INFECTION

Recent analysis of the kinetics and the composition of the PBA phenomenon in mice infected with the lethal variant of **P.yoelii** allows us to conclude that the formation of some hetero-Abs during the infection cannot be accounted by PBA alone (Burger, Daniel-Ribeiro & Ballet, submitted) . Mice were infected with lethal **P.yoelii** or injected with hemolysates or plasmas from **P.yoelii** infected mice or supernatants from **in vitro P. falciparum** cultures. Parameters such as the spleen/total body weights ratio, the number of nucleated spleen cells, the percentage of Ig contaning (IgCC) and of Ig secreting cells (IgSC), evaluated respectively by direct immunofluorescence and by the reverse haemolytic plaque assay, rose progressively, paralleling the parasitaemia, until day 18 of infection when the parasitaemia reached its maximum values.

A different pattern of kinetics was observed when the number of specific (anti-sheep red blood cell-SRBC and anti-trinitrophenyl-TNP) responses were evaluated. In those cases an early peak of responses was recorded at day 4 (anti-SRBC PFC) or day 9 (anti-TNP PFC) when the PBA, reflected by the increase in the number of total IgSC, was still unapparent, the response decreased thereafter and were back to preinfection values at day 18 when the PBA showed its highest values.

Moreover the fact that, the limited effects (a 2.5 to 4 fold increase) of injection of extracts thought to contain parasite derived substances in the total numbers of IgCC and IgSC could not account for the 10 to 30 fold increase in the numbers of SRBC-specific cells, suggested that the formation of the hetero-Abs during malaria was not a direct consequence of the PBA phenomenon but should rather be interpreted as a "preferential" (Ag induced) activation of these Ag specific cells. It is suggested that SRBC specific Ab appear as a result of cross reactivities between this Ag and parasite modified host Ags (as that existing between SRBC and bromelain treated mouse RBC, Pages & Bussard, 1975), that the PBA taking place during the infection appear as a result of sucessives waves of Ag-specific B-cell activation and that the idiotype-anti-idiotype framework could participate in its genesis.

PBA AND ANAEMIA IN MALARIA INFECTION

Human **Plasmodium** are obligate intracellular parasites that infect and destroy RBC during their cycle in the vertebrate host. Malaria infection is therefore usually acccompained by a variable degree of anaemia that, however, does not correlate with the degree of parasitaemia and often appears when no more parasites can be detected in the circulating blood. These facts suggest that immunological factors can participate in its genesis.

In fact, besides mechanical rupture or an anti-plasmodial Ab dependent lysis of infected RBC (reviewed by Seed & Kreier, 1980) several immunological mechanisms could operate in the production of the malaria anaemia. Among the evoked mechanisms is the destruction of RBC by anti-erythrocyte auto-Ab or by adsorbed immune complexes induced by PBA and it is known that both situations are associated with PBA (Hammarstrom et al., 1976; Lambert et al., 1983). In order to investigate the role of the phenomenon of PBA and of RBC sensitization by Ig and complement in the induction of malaria anaemia we studied the relationship between the degree of PBA, that of RBC sensitization and that of anaemia in 138 malaria infected (MI) and 49 non infected (NMI) individuals from an endemic area of malaria in the northwest of Brazil (Ariquemes - Rondonia) (Daniel Ribeiro et al., 1986).

Patients were assessed by the degree of activation of Ig (G, A, M) secreting cells (IgSC) by a reverse haemolytic plaque assay, for the degree of RBC sensitization by a sensitive immunoradiometric assay and for the anaemia. The numbers of activated IgGSC and of IgMSC were found to be significantly increased in MI when compared to NMI, the same was true for the amount of RBC associated IgG (but not IgM or C3d). The degree of anaemia was not related to the parasitaemia but was positively and significantily related

to the degree of IgG sensitized RBC. This increase in the amount of IgG RBC was not related to the increase in the numbers of IgGSC suggesting that although the sensitization of erythrocytes by IgG molecules can be involved in the pathogenesis of the malaria anaemia it does not seem to be a direct consequence of the malaria associated PBA phenomenon.

CAN PBA REPRESENT A HANDICAP TO THE DEVELOPMENT OF ANTI SPOROZOITE IMMUNITY?

It has been known for a long time that the administration of a B-cell mitogen such as the lipopolysacaride of **E. coli** to mice (therefore inducing a PBA status) before the injection of a given Ag can suppress the specific response to this Ag (Diamantstein et al., 1976). In addition, the course of infections due to microorganisms endowed with PBA properties (such as malaria and American or African Trypanosomiasis) is accompanied by immunossuppression to several Ags (for review see Daniel Ribeiro, 1983).

In order to study the relevance of the malaria associated PBA in the development of anti-sporozoite specific immunity we used a reversal haemolytic plaque assay and an immunoradiometric assay employing the synthetic peptide (NANP)3, the main epitope of CS protein of **P. falciparum**, to assess respectively the degree of activation of IgG and IgM secreting cells and the level of anti-sporozoite Abs in 95 malaria infected and 21 non infected individuals in the Nortwest Brasil (Daniel-Ribeiro, Oliveira-Ferreira, Banic & Galvao-Castro, submitted) . A positive correlation was observed between the anti-(NANP)3 Ab levels and the number of past attacks of malaria but not between the former and the age of individuals or the numbers of months of residence in the region. Individuals with high numbers of IgG or of IgMSC presented lower anti-(NANP)3 Ab levels and conversely those with levels of Ab above the mean level calculated for malaria infected individuals showed lower numbers of IgGSC and higher haematocrit and haemoglobin values.

Three hypothesis were considered to explain this negative relationship between PBA and anti-(NANP)3 Ab levels. The first postulates that PBA and low responsiveness to sporozoite antigens would be only markers for a third unrelated factor that would determine the ability of B-cells to be activated during malaria infection, the second hypothesis considers that individuals with higher levels of anti-(NANP)3 Ab would be more protected against malaria and consequently more protected against the malaria associated PBA. In this regard it must be enphasized that anti-(NANP)3 Ab positive individuals presented numbers of IgMSC and of IgGSC and haematocrit and haemoglobin values similar to those registered for non infected individuals. Finally the third hypothesis is that, by some mechanism, individuals

with high degrees of PBA could be less able to elaborate an effective anti-sporozoite immune response. This mechanism is illustrated by the finding by Corsini & Costa (1981a and b) that a crude extract of T. **cruzi** trypomastigotes, that mimicked the PBA effects of the infection, could suppress the humoral immune response to specific Ag when injected prior to the Ag injection. One additional argument to support this hypothesis came with the observation by Orjih & Nussenzweig (1979) that acute blood stage induced **P. berghei** infection (a situation known to be associated with PBA - Freeman & Parish, 1978) can suppress the production of anti-sporozoite antibodies of **P. berghei**.

If the last hypothesis is correct and since we have recently observed (Banic, Viana-Martins, de Souza & Daniel- Ribeiro. Polyclonal B-lymphocyte activation in human malaria, manuscript in preparation) that the PBA recorded in parasitized individuals disappeared in a 10 days period after the treatment was started, chemotherapic measures should be considered before the initiation of malaria immunoprophylatic campaigns in populations chronically exposed to the risk of malaria infection.

CONCLUSIONS

Taken together the data reviewed here suggest that malaria infection is accompanied by a marked degree of PBA that can be related to the existence of parasite derived T-cell mitogens. At least in some cases, the malaria associated PBA can participate in the origin of auto and hetero-Ab production during the infection by furnishing (or potentiating) one of the signals required for B-cell activation. It could also be involved in the immunosuppression observed in some instances during malaria infection but does not seem to be directely implicated in the genesis of the malaria anaemia.

ACKNOWLEDGEMENTS

Dr.C.Daniel-Ribeiro is receipient of a grant from the CNPq (Brazil).The author thanks Mss. Joseli de Oliveira Ferreira, Helena Cristina Balthazar Guedes and Maria da Gloria Mangueira Este for preparing and Dr. Helio G. Pereira for critically reviewing the manuscript.

REFERENCES

ABELE, D. C. , TOBIE, J. E. HILL, G.J., CONTACOS, P.G. & EVANS, C.B. 1965. Alterations in serum proteins and 19S antibody production during the course of induced malarial infections in man. Am. J. Trop. Med. Hyg.,14, 191-197.

ADENYI-JONES, C. 1967. Agglutination of tanned sheep erythrocytes by serum from Nigerian adults and children. Lancet.,1, 188-190.

ADU, B., GWYN WILLIAMS, D., QUAKYI, I.A., VOLLER, A., ANIM-ADDO, Y., BRUCE-TAGOE, A.A., JOHNSON, G.D. & HOLBOROW, E.J. 1982. Anti-ssDNA antinuclear antibodies in human malaria. Clin. Exp. Immunol.,49, 310-316.

BALLET, J.J., DRUILHE, P., QUERLEUX, M.A., SCHMITT, C. & AGRAPART, M. 1981. Parasite-derived mitogenic activity for human T cells in Plasmodium falciparum continous cultures. Inf. Immun.,33, 758-762.

BANKHURST, A.D., TORRIGIANI, G. & ALLISON, A.C. 1973. Lymphocytes binding human thyroglobulin in healthy people and its relevance to tolerance for autoantigens. Lancet,1, 226-230.

BANKHURST, A.D. & WILLIANS, R.C. 1975. Identification of DNA-binding lymphocytes in patients with systemic lupus erythematosus. J. Clin. Invest.,56, 1378-1385.

BEALL, G.N. & KRUGER, S.R. 1980. Production of human antithyroglobulin in vitro I. Stimulation by mitogens. Clin. Immunol. Immunopathol.,16, 485-497.

BIRD, A.G., HAMMARTROM, L., SMITH, C.I.E. & BRITTON, S. 1981. Polyclonal human T lymphocyte activation results in the secondary functional activation of the human B lymphocytes. Clin. Exp. Immunol.,43, 165-173.

BRETSCHER, P. & COHN, M. 1970. A theory of self-nonself discrimination. Science,169, 1042-1049.

BURGER,L., DANIEL-RIBEIRO,C. & BALLET,J.J. Kinetics and composition of polyclonal B-cell activation during lethal **P.yoelii** (17 XL) infection in CBA/J and swiss OF 1 mice. (submitted)

CHESUD, T.M., STEINBERG, A.D. & TALAL, N. 1972. The clearance and localization of nucleic acids by New Zealand and normal mice. Clin. Exp. Immunol.,12, 465.

COHN, M. & BLOMBERG, B. 1975. The self-nonself discrimination: a one or two signal mechanism? Scand. J. Immunol.,4, 1-24.

CORSINI, A.C. & COSTA, M.G. 1981a. Immunosuppression in mice infected with Trypanosoma cruzi (Chagas, 1909): I evidences of polyclonal B cell activation in experimental infections mimicked by an extract prepared from circulating trypomastigotes. Rev. Inst. Med. Trop. Sao Paulo,23, 114-121.

CORSINI, A.C. & COSTA, M.G. 1981b. Immunosuppresion in mice infected with Trypanosoma cruzi (Chagas, 1909). II Trypomastigote crude extract (TCE) suppress the humoral immune response in mice. Rev. Inst. Med. Trop. Sao Paulo,23, 122-126.

COUTINHO, A. & MOLLER, G. 1974. Immune activation of B cells: Evidence for "one nonspecific triggering signal" not delivered by the Ig receptors. Scand. J. Immunol.,3, 133-146.

CUNNINGHAM, A.J. 1976. Self-tolerance maintained by active suppressor mechanisms. Transplant. Rev.,31, 23-43.

CURTAIN, C.C., KIDSON, C. CHAMPNESS, D.L. & GORMAN, J.G. 1964. Malaria antibody content of gamma 2-7S globulin in tropical populations. Nature,203, 1366-1367.

DANIEL RIBEIRO, C., MONJOUR, L., PONTES DE CARVALHO, L. C., PLAYFAIR, J.H.L. & GENTILINI, M. 1982. Malaria infection and simultaneous administration of mouse thyroglobulin induce a specific autoimmune response in mice. Mol. Biochem. Parasitol., supplement, 365-366, Fifth Int. Congress Parasitol. Toronto.

DANIEL RIBEIRO, C. 1983. L'activation polyclonale des cellules-B, l'autoimmunite et l'immunosuppression au cours du paludisme. These Doctorat d'Etat, Universite Paris IV.

DANIEL RIBEIRO, C. , DRUILHE, P., MONJOUR, L., HOMBERG, J.C. & GENTILINI, M. 1983a. Specificity of auto-antibodies in malaria and the role of polyclonal activation. Trans. Roy. Soc. Trop. Med. Hyg.,77, 185-188.

DANIEL RIBEIRO, C., TIRARD, D., MONJOUR, L., HOMBERG, J.C. & GENTILINI, M. 1983b. Relevance of autoantigens to autoimmunity in African Trypanosomiasis: study of DNA and thyroglobulin antibodies. Acta Trop.,40, 321-329.

DANIEL RIBEIRO, C., ALFRED, C., MONJOUR, L. & GENTILINI, M. 1984a. Normal frequency of anti-thyroglobulin antibodies in hyperendemic areas of malaria: relevance to the understanding of autoantibody formation in malaria. Trop. Geogr. Med.,36, 323-328.

DANIEL RIBEIRO, C.T., DRUILHE, P., de ROQUEFEUIL, S., MONJOUR, I., HOMBERG, J.C. & GENTILINI, M. 1984b. Abnormal anti-single stranded (ss) DNA activity in sera from Plasmodium falciparum infected individuals. Trans. Roy. Soc. Trop. Med. Hyg.,78, 742-746.

DANIEL RIBEIRO, C., BANIC, D.M., AHMED, I.I. & GALVAO-CASTRO, B. 1986. Polyclonal B-lymphocyte activation and sensitization of erythrocytes by IgG in human malaria: Relevance to the development of anaemia in a holoendemic area in Northwestern Brazil (Ariquemes - Rondonia). Mem. Inst. Oswaldo Cruz, Rio de Janeiro,81, Suppl. II, 169-176. Intern. Symp. on Malaria, Rio de Janeiro.

DANIEL RIBEIRO,C., OLIVEIRA-FERREIRA,J., BANIC,D. & GALVAO-CASTRO,B. Can malaria associated polyclonal B-lymphocyte activation interfere with the development of anti-sporozoites specific immunity? (Submitted)

DIAMANTSTEIN, T., KEPPLER, W., BLITSTEIN-WILLINGER, E. & BENEFRAIM. 1976. Suppresion of the primary immune response in vivo to sheep red blood cells by B-cell mitogens. Immunol.,30, 401-407.

ESQUIVEL, P.S., ROSE, N.R. & KONG, Y.C.M. 1977. Induction of autoimmunity in good and poor responder mice with mouse thyroglubulin and lipopolysaccharide. J. Exp. Med.,145, 1250-1263.

FALANGA, P.B., D'IMPERIO LIMA, COUTINHO, A.& Pereira da Silva, L. 1987. Isotypic pattern of the polyclonal B-cell response during primary infection by **Plasmodium chabaudi** and in immune-protected mice. Eur. J. Immunol.17, 599-603.

FOURNIE, G.J., LAMBERT, P.H. & MIESCHER, P.A. 1974. Release of DNA in circulating blood and induction of anti-DNA antibodies after injection of bacterial lipopolysaccharides. J. Exp. Med.,140, 1189-1205.

FREEMAN, T., SMITHERS, S.R., TARGETT, G.A.T. & WALKER, P.J. 1970. Specificity of immunoglobulin G in Rhesus monkeys infected with Schistosoma mansoni, Plasmodium Knowlesi and Trypanosoma brucei. J. Inf. Dis.,121, 401-406.

FREEMAN, R.R. & PARISH, C.R. 1978. Polyclonal B-cell activation during rodent malarial infections. Clin. Exp. Immunol.,32, 41-45.

GREENWOOD, B.M. 1970. Heterophile antibodies in Nigerian sera. Clin. Exp. Immunol.,6, 197-206.

GREENWOOD, B.M., HERRICK, E.M. & HOLBOROW, E.J. 1970. Speckled antinuclear factor in African sera. Clin. Exp. Immunol.,7, 75-83.

GREENWOOD, B.M., MULLER, A.S. & VALKENBURG, H.A. 1971. Rheumatoid factor in Nigeria sera. Clin. Exp. Immunol.,8, 161-173.

GREENWOOD, B.M. 1974. Possible role of a B-cell mitogen in hypergammaglobulinaemia in malaria and trypanosomiasis. Lancet,1, 435-436.

GREEENWOOD, B.M. & VICK, R. 1975. Evidence for a malaria mitogen in human malaria. Nature,257, 592-594.

GREENWOOD, B.M., ODULOJU, A.J. & PLATTS-MILLS, T.A.E. 1979. Partial characterization of a malaria mitogen. Trans. Roy. Soc. Trop. Med. Hyg.,73, 178-182.

HAMMARSTROM, L., SMITH, E., PRIMI, D. & MOLLER, G. 1976. Induction of autoantibodies to red blood cells by polyclonal B-cell activators. Nature,263, 60-61.

HOUBA, V. & ALLISON, A.C. 1966. M-antiglobulins (rheumatoid-factor-like globulins) and other gamma-globulins in relation to tropical parasitic infections. Lancet.,1, 848-852.

HOUBA, V., PAGE-FAULK, W. & MATOLA, Y.G. 1974. Heterophilic antibodies in relation to malarial infection: Population and experimental studies. Clin. Exp. Immunol.,18, 89-93.

IZUI, S., ZALDIVAR, N.M., SCHER, I. & LAMBERT, P.H. 1977. Mechanism for induction of anti-DNA antibodies by bacterial lipopolysaccharides in mice. I. Anti-DNA induction by LPS without significant release of DNA in circulating blood. J. Immunol.,119, 2151-2156.

JAYAWARDENA, A.N., TARGETT, G.A.T., LEUCHARS, E., CARTER, R.L., DOENHOFF, M.J. & DAVIES, A.J.S. 1975. T-cell activation in murine malaria. Nature,258, 149-151.

KANO, K.K., McGREGOR, I.A. & MILGRON, F. 1968. Hemagglutinins in sera of Africans of Gambia. Proc. Soc. Exp. Biol. (N.Y.),129, 849-853.

KHOURY, E.L., RITACCO, V., COSSIO, P.M., LAGUENS, R.P., SZARFMAN, A., DIEZ, C. & ARANA, R.M. 1979. Circulating antibodies to peripheral nerve in American trypanosomiasis (Chagas disease). Clin. Exp. Immunol.,36, 8-15.

KREIER, J.P. & DILLEY, D.A. 1969. Plasmodium berghei: nucleic acid agglutinating antibodies in rats. Exp. Parasitol.,26, 175-180.

LAMBERT, P.H., MOREL, P.A., RENVERSEZ, J.C. & GOLDMAN, M. 1983. Idiotypic interactions and immune complexes in infectious diseases. In Progress in Immunology V.Y. Yamamura & T. Tada, eds., Academic Press, New York, 1343.

LANGHORNE, J., KIM,K.J. & ASOFSKY, R. 1985. Distribution of Immunoglobulin isotypes in the nonspecific B-cell response induced by infection with **Plasmodium chabaudi adamiand Plasmodim yoelii**. Cell. Immunol., 90,251-257.

LEFRANCOIS, G., LE BRAS, J., SIMONNEAU, M., BOUVET, E., VROKLANS, M. & VANCHON, F. 1981. Anti-erythrocyte autoimmunisation during chronic falciparum Malaria. Lancet,2, 661-664.

LINDSLEY, H.B., KYSELA, S. & STEIBERG, A.D. 1974. Nucleic acid antibodies in African trypanosomiasis: studies in Rhesus monkey and man. J. Immunol.,113, 1921-1927.

NAKASHIMA, I., YOKOCHI, T., KATO, N. & ASAI, J. 1977. Microbial adjuvant and autoimmunity. II. Production of lesions in mice immunized with syngeneic tissue extracts together with the capsular polysaccharide of Klebsiella pneumoniae. Microbiol. Immunol.,21, 279-288.

ORJIH, A.U. & NUSSENZWEIG, R.S. 1979. Plasmodium berghei: suppression on antibody response to sporozoite stage by acute blood stage infection. Clin. Exp. Immunol.,38, 1-8.

OSUNKOYA, B.O., WILLIAMS, A.I.O. & REDDY, S. 1972. Spontaneous lymphocyte transformation in leucocyte cultures of children with Falciparum Malaria. Trop. Geogr. Med.,24, 157-161.

PAGES, J. & BUSSARD, A.E. 1975. Precommitment of normal mouse peritoneal cells by erytrocyte antigens in relation to autoantibody production. Nature,257, 316-317.

POELS, L.G. & VAN NIEKERK, C.C. 1977. Plasmodium berghei. Immunosuppression and hyperimmunoglobulinemia. Exp. Parasitol.,42, 235-247.

POELS, L.G., VAN NIEKERK, C.C. & JERUSALEM, C. 1978. Glomerulopathy in mice infected with Plasmodium berghei. Induction of an autoimmune process. Israel J. Med. Sci.,14, 651-654.

POELS, L.G., VAN NIEKERK, C.C., VAN DER STERREN-RETI, V. & JERUSALEM, C. 1980. Plasmodium berghei: T-cell-dependent autoimmunity. Exp. Parasitol.,49, 97-105.

PRIMI, D., HAMMARSTROM, L., EDVARD SMITH, C.I. & MOLLER, G. 1977a. Characterization of self-reactive B-cells by polyclonal B-cell ativators. J. Exp. Med.,145, 21-30.

PRIMI, D., SMITH, C.I.E., HAMMARSTROM, L., LUNDQUIST, P.G. & MOLLER, G. 1977b. Evidence for the existence of self reactive human B lymphocytes. Clin. Exp. Immunol.,29, 316-319.

QUAKYI, I.A., VOLLER, A., HALL, A.P., JOHNSON, G.D., HOLBOROW, E.J. & MOODY, A.H. 1979.Immunological abnormalities in causasians with malaria. Immunology letters,1, 153-154.

ROBERTS, I.M., WHITTINGHAM, S. & MACKAY, I.R. 1973. Tolerance to an autoantigen-thyroglobulin. Lancet.,2, 936-940.

ROSENBERG, Y.J. 1978. Autoimmune and polyclonal B-cell response during murine malaria. Nature,274, 170-172.

SEED, T.M. & KREIER, J.P. 1980. In Kreier J. P. ed., Malaria, vol.2. Patholy vector studies and culture, 1-46, Academic Press, N.Y.

SHAPER, A.G., KAPLAN, M.H., MODY, N.J. & Mc INTYRE, P.A. 1968. Malarial antibodies and autoantibodies to heart and other tissue in the immigrant and indigenous peoples of Uganda. Lancet.,1, 1342-1347.

SHOENFELD, Y., RAUCH, J., MASSICOTTE, H., DATTA, S.K., ANDRE-SCHWARTZ, J., STOLLAR, D. & SCHWARTZ, R.S. 1983. Polyspecificity of monoclonal lupus antibodies produced by human-human hybridomas. N. Eng. J. Med.,1, 414-420.

STRICKLAND, G.T. 1978. Lymphotic mitogenic factor in sera from patients with falciparum malaria. Tropenned. Parasit.,29, 198-203.

TEODORESCU, M. 1981. Characterization and role in autoimmune diseases of the polyclonal B-cell activator produced by T-cells. The helper factor. Immunol. Rev.,55, 155-178.

VIAL, H.J., THUET, M.J., BROUSSAL, J.L. & PHILIPPOT, J.R. 1982. Phospholipid biosynthesis by Plasmodium knowlesi-infected erythrocytes: the incorporation of phospholipid precursors and the identification of previously undetecd metabolic pathaways. J. Parasitol.,68, 379-391.

VOLLER, A., O'NEILL, P. & HUMPHREY, D. 1972. Serological indices in Tanzania II. Antinuclear factor and malarial indices in populations living at different altitudes. J. Trop. Med. Hyg.,75, 136-139.

WEINBAUM, F.I., EVANS, C.B. & TIGELAAR, R.E. 1976. An in vitro assay for T cell immunity to malaria in mice. J. Immunology,116, 1280-1283.

WEINBAUM, F.I., WEINTRAUB, J., NKRUMAH, F.K., EVANS, C.B., TIGELAAR, R.E. & ROSENBERG, Y.J. 1978. Immunity to Plasmodium berghei yoelii in mice. II. Specific and non specific cellular and humoral responses during the course of infection. J. Immunol.,121, 629-636.

WYLER, D.J. & OPPENHEIM, J.J. 1974. Lymphocyte. Transformation in human Plasmodium falciparum malaria. J. Immunol.,113, 449-454.

WYLER, D.J., HERROD,H.G. & WEINBAUM, F.I. 1979. Response of sensitized and unsensitized human lymphocyte subpopulations to Plasmodium falciparum antigens. Infects. & Immun.,24, 106-110.

ZOUALI, M., DRUILHE, P. & EYQUEM, A. 1986. IgG-subclass expression of anti-DNA and anti-ribonucleoprotein autoantibodies in human malaria. Clin. Exp. Immunol.,66, 273-278.