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POLYCLONAL B-CELL ACTIVATION IN HUMAN MALARIA: RELEVANCE TO THE DEVELOPMENT OF ANTI-SPOROZOITE SPECIFIC IMMUNE RESPONSE AND OF IMMUNOPATHOLOGY IN INDIVIDUALS FROM ENDEMIC AREAS (RONDONIA STATE — BRAZIL)

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RELEVANCE OF POLYCLONAL B-CELL ACTIVATION (PBA) TO THE DEVELOPMENT OF THE MALARIA ANAEMIA.

Malaria is caused by an obligate intracellular parasite that infect and destroy red blood cells (RBC) during its cycle in the vertebrate host. The infection is therefore accompained by a variable degree of anaemia that however does not correlate with the degree of parasitaemia in the infected host, fact that suggest the participation of immunological factors in the genesis of the malaria associated anaemia.

Besides to the mechanical rupture and an anti-plasmodial antibody (Ab) dependent lysis of infected RBC (for review see reference[12]) and a mild degree of bone marrow depression[13], several immune mechanisms could operate in the production of the anaemia[14]. One of the evoked mechanisms is the formation of anti-erythrocyte Auto-Abs that could appear as a result of either cross-reactions between parasites and RBC or polyclonal activation of autoreactive clones of B-lymphocytes specific to RBC antigens[8].

Indeed it is now clear that polyclonal B-cell activation or activators can lead to Auto-Ab production[8] and that Plasmodia are endowed with PBA properties[1-11]. Moreover PBA can induce the formation of immune complexes that could adhere to the RBC membrane[9].

In order to investigate the role of PBA and of RBC sensitization by immunoglobulins (IgG and IgM) and complement in the malaria induced anaemia we studied 138 malaria infected (MI) and 49 non infected (NMI) individuals for the: a) degree of PBA by a reverse haemolytical plaque assay able at detecting Ig synthesis of any specificity; b) the degree of RBC sensitization by a sensitive immunoradiometric assay employing class-specific anti immunoglobulins and iodinated protein A and c) the anaemia by classical haematological procedures. Patients were seen at the out patient clinics of SUCAM or hospitalized in the hospital unit of the Fundação SESP both in the town of Ariquemes (Rondonia-State). Control individuals were assymptomatic local inhabitants without history of previous malaria. This part of the work has been published elsewhere[5], in this paper we will only summarize the main results and conclusions of this study.

Polyclonal B-cell activation, as reflected by an increase in the numbers of IgG and IgM secreting cells (IgSC) in the peripheral blood, was

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observed in both **P. vivax** and **P. falciparum** MI but not in NMI. Although the numbers of IgASC were not above the normal figures a positive and significant relationship was observed between these numbers and those of IgMSC and of IgGSC in both cases of Plasmodial infection. In addition, the degree of PBA was positively related to that of parasitaemia but no relationship could be detected between the former and the number of past attacks of malaria (PAM). In the same way no difference was observed in the number of IgSC (G, A or M) between males and females or those above and below thirty (the mean age of the studied population).

The percentages of IgG (but not of IgM or C3d) sensitized RBC were significantly higher in MI than in NMI and a positive relationship was observed between the numbers of IgM-RBC and those of IgG-RBC and between those of IgM and of C3d-RBC. No relationship was however observed between the amount of sensitized RBC and the parasitaemia.

Malarious individuals presented low haematocrite and haemoglobin values, however no relationship could be observed between these values and the parasitaemia. In order to study the role of PBA in the RBC sensitization and the malaria associated anaemia we study the relationship between: a) the degrees of PBA and of RBC sensitization; b) the levels of PBA and of anaemia and c) the degrees of RBC sensitization and that of anaemia. No relationship could be observed between the degree of activation of Ig secreting cells of a given class and the sensitization of RBC by the same class of Ig showing that the PBA does not seem to be directly involved in the RBC sensitization by immunoglobulin. In **P. vivax MI** a significant and positive relationship was observed between the amount of IgG sensitized RBC and the degree of anaemia and although such a relationship could not be observed in **P. falciparum MI** the haematocrite values were significantly lower in **P. falciparum MI** with high levels than in those with low levels of IgG-RBC.

Taken together these data suggest that although the PBA phenomenon can be excluded as the sole cause of Ig sensitization of RBC, and of anaemia the IgG sensitization of RBC could be involved in pathogenesis of the malaria associated anaemia. Whether these Immunoglobulins are indeed anti-erythrocyte auto-antibodies or are in fact Abs complexed to malarial antigens passively adsorbed to the RBC membrane is a matter of study in our laboratory but our preliminary results seems to indicate that the second hypothesis is more likely.

CNA PBA INTERFERE WITH THE DEVELOPMENT OF ANTI-SPOROZOITE IMMUNITY?

It has been known for a long time that the administration of PBA such as the lipopolysaccharide of **E. coli** to mice, situation associated to a polyclonal B-cell activation status, before the injection of a given antigen can suppress the immune response to this antigen[3] in addition, diseases caused by parasites endowed with PBA properties such as American or African trypanosomiasis, (for review see **4**) are also associated to an immunosuppression to several antigens.

Therefore it should be of interest to evaluate whether or not the malaria associated PBA phenomenon could represent a handicap to the development of an effective anti-sporozoite immunity.

In order to study this point 95 malaria infected and 21 non infected individuals from the town of Ariquemes (Rondonia-State) in the north-west of Brazil were assessed for the percentage of IgG and IgM secreting cells in the peripheral blood and for the level of anti-sporozoite antibodies (by a immunoradiometric assay using the (NANP)3 synthetic peptide that corresponds to the immunodominant epitope of the CS protein of the sporozoite membrane). The results of this study are been published elsewere[6], but, once more, here we will summarize its main results and conclusions.

A significant and positive relationship was observed between the anti-(NANP)3 Ab levels and the number of past attacks of malaria (PAM) but not between the former and the age or the time of residence of individuals in the region. Individuals with high numbers of IgGSC or IgMSC presented lower levels of anti-(NANP)3 Ab and conversely those with levels of anti-

(NANP)3 Ab above the main level calculated by MI showed normal values of IgGSC and of IgMSC as well as of haematocrite and haemoglobin.

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 would be only marker for a third unrelated (intrinsic or extrinsic) 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 PBA induced by malaria. In this regard it should be emphasized that anti-(NANP)3 Ab positive individuals had numbers of IgGSC and of IgMSC as well as haematocrite and haemoglobin values similar to those registered for non infected individuals. Finally the third hypothesis postulates an inverse cause-effect relationship, i.e. 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[2, 3] that a crude extract of **T. cruzi** tyrpomastigotes, that mimicked the PBA properties of the infection, could suppress the immune response to the specific antigen when injected prior to the Ag injection. One additional evidence to this mechanism is the observation that blood stage induce **P. berghei** infection a situation known to be associated with PBA[11] can suppress the production of anti-sporozoite antibodies in mice vaccinated with irradiated sporozoites[10].

If the last hypothesis is correct, and since we have recently observed that the malaria associated PBA disappeared in a 10 days period after the treatment was started, chemotherapeutic measures should be considered before any immunoprophylatic campaing are initiated in populations chronically exposed to the risk of malaria infection.

CONCLUSIONS

The results overviewed here seem to indicate that: a) a polyclonal B-cell activation, reflected by an increase in the number of immunoglobin (IgG and IgM) secreting cells in the peripheral blood, exists in malaria infected individuals, b) this PBA status does not seem to be involved in the RBC sensitization by Ig or in the malaria associated anaemia but could affect the anti-sporozoite specific immune response and c) the RBC sensitization by IgG could participate in the genesis of the malaria anaemia in **P. vivax** and even, in a less extent, in **P. falciparum** infected individuals.

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