Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87*, Suppl. IV, 167-170, 1992

EFFECTOR FUNCTIONS OF EOSINOPHILS IN SCHISTOSOMIASIS

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The dual function of eosinophils is clearly illustrated in schistosomiasis. Well equipped in membrane receptors for immunoglobulins and complement, and due to the presence of granule basic proteins, eosinophils can becone cytotoxic for parasite larvae and thus participate to protective immunity. However mediators can also exert their cytolytic effect on normal cells or tissues, inducing therefore pathology. Through ADCC mechanisms against schistosome larvae in vitro *involving different antibody isotypes (IgG, IgE and IgA) and also in experiments performed* in vivo, *eosinophils have been clearly involved in protective immunity.*

Although no direct evidence of the protective role of eosinophils were brought in humans, the striking association of eosinophil-dependent cytotoxic antibody isotypes with resistance to reinfection (for instance IgE and IgA antibodies), whereas in vitro *blocking antibody isotypes (IgG4, IgM) were detected in susceptible subjects, strongly, suggested the participation of eosinophils in antibody-dependent protective immune response. However eosinophils could also participate to granuloma formation around* S. mansoni *eggs and consequently to the pathological reactions induced by schistosomiasis.*

Key words: eosinophils – schistosomiasis – immunoglobulins – isotypic selection – protection – tissue damage

The effector function of eosinophils has been discovered in schistosomiasis and fully documented in numerous studies performed both *in vitro* and *in vivo*. The presence of a large variety of membrane receptors and the identification of cytotoxic molecules have allowed, in the recent years, to consider eosinophils as effector cells, able, in the presence of specific antibodies, to kill parasite targets and specially schistosomula. Concerning membrane receptors, eosinophils express receptors for the Fc fragment of immunoglobulins, namely Fc gamma receptor (Fcγ RII/CD32), Fc epsilon receptor (Fcε RII/CD23) and Fc alpha receptor (Fcα R). They exhibit receptors for complement fragments, CR1 (CD35), CR3 (CD11b), as well as receptors for C3a, C5a, or C1q. Receptors for chemotactic factors (PAF-acether, LTB4, ECFA) have been also described. More recently, the existence of receptors for interleukins has been demonstrated on human eosinophils (receptors for IL-3, GM-CSF, IL-2 and IL-5). In addition, eosinophils possess several adhesion molecules such as LFA-1 alpha (CD11a), P150-95 (CD11c), the common P95 beta chain (CD18) and more recently the VLA-4 molecule (CD49d, CD29). The presence on eosinophils, but not on neutrophils, of VLA-4, which binds to its specific ligand on endothelial cells, might explain the specific tissue infiltration of eosinophils in some diseases, such as parasitic infections or allergic states.

Eosinophils are characterized by the presence of specific granules: the biochemical content of these granules is now well known. The central core or crystalloïd is composed of one protein, the Major Basic Protein (MBP) whereas the matrix of the granules contains Eosinophil Cationic Protein (ECP), Eosinophil Derived Neurotoxin (EDN) and Eosinophil Peroxidase (EPO). All these proteins, highly basic, are the major factors responsible for the cytolytic properties of eosinophils, not only against parasite targets but also against normal mammalian cells or tissues. Besides these basic proteins, the granules also contain several lysosomal enzymes. Eosinophils are able to generate newly formed mediators such as LTC-4, PAF, PGE-2, Substance P or VIP (VasoIntestinal Peptide). Very interestingly, it has been

discovered that eosinophils not only produced cytotoxic or inflammatory mediators but also cytokines, such as IL-1, TGF-alpha, IL-3 and GM-CSF. The granule basic proteins participate in the dual function of eosinophils: when their cytotoxic properties are directed against non self targets such as parasites, tumor cells or bacteria, eosinophils can be involved in a protective immune response. However, when the cytolytic properties are directed against normal mammalian cells or tissues such as epithelial cells, lung cells, nervous cells, or cardiac cells for instance, eosinophils participate to the pathology.

Eosinophils are able to kill schistosomula targets in the presence of specific antibodies, in a classical Antibody Dependent Cellular Cytotoxicity (ADCC) mechanism. This mechanism has been demonstrated not only in humans but also in the case of rat or monkey eosinophils. It requires an initial step of adherence of eosinophils to their targets. This adhesion is due first to the binding of specific antibodies to parasite surface through their Fab fragments and to eosinophil Fc receptor via their Fc fragments. However, besides this requirement, it has been shown that other eosinophil surface molecules could be involved. In the case of IgE dependent cytotoxicity, not only FcεRII but also CR3 are required, as shown by inhibition experiments with monoclonal antibodies directed against CR3, which inhibited both adherence and cytotoxicity whereas monoclonal antibodies anti-CR1 had no inhibitory effect (Capron et al., 1987b).

In the past years, the IgE receptor of human eosinophils has been extensively studied and recently reviewed (Capron et al., 1989). It has been recently suggested that this IgE receptor (FcεRII) cross-reacted with the CD23 molecule (differentiation marker of B cells): 3 different anti-CD23 monoclonal antibodies were able to decrease, in a dose-dependent manner, the IgE binding and the IgE-dependent cytotoxicity of human eosinophils for schistosomula targets (Capron et al., 1991b). However, the CD23 molecule does not represent the only IgE binding protein expressed by eosinophils, since we could show that a molecule, belonging to the family of carbohydrate binding proteins was also involved in IgE-dependent effector function of eosinophils (in preparation).

Human eosinophils purified from hypereosinophilic patients are highly heterogeneous and it has been possible to fractionate subpopulations of eosinophils differing by their density: "hypodense" eosinophils with an abnormally low density which represent the activated phenotype, in contrast to "normodense" eosinophils with a normal density, which are less activated. Very interestingly, we have demonstrated that hypodense eosinophils were significantly more cytotoxic for schistosomula in the presence of IgE antibodies than normodense eosinophils (Capron et al., 1989). Among the various factors responsible for this activation such as chemotactic factors, the role of interleukins has been recently investigated and it has been shown that IL-5, GM-CSF and TNF alpha were able to increase in a dose-dependent manner the IgE dependent cytotoxicity of human eosinophils, whereas IL-4 and interferon gamma had no significant effect (Capron et al., 1991a). These results associated to other studies on the effects of interleukins on eosinophil activation and survival *in vitro,* suggested that hypodense eosinophils have probably been activated by interleukins *in vivo.*

Besides these studies on the effector cell, we have investigated the interactions between eosinophils and antibodies. A large series of experiments have demonstrated, first in the rats and now in humans that not all the antibody isotypes could induce eosinophil cytotoxicity, leading to the concept of isotypic selection. Whereas initial studies performed in rats had clearly shown the existence of one major cytotoxic isotype (IgG2a), and one potent blocking isotype (IgG2c), similar results have been obtained in the case of human antibodies. First, it was shown that IgM antibodies present in the serum of schistosomiasis patients and depleted of IgG antibodies by absorption onto Protein A-Sepharose, were able to decrease eosinophil-dependent cytotoxicity mediated by IgG antibodies (Khalife et al., 1986). IgM antibodies could therefore be considered as blocking antibodies in human serum. Concerning IgG antibodies, it was shown thereafter that IgG1 and IgG3 antibodies were the more potent isotypes to induce cytotoxicity, whereas IgG2 antibodies were only cytotoxic in the presence of activated eosinophils. In contrast, IgG4 antibodies were able to inhibit the cytotoxicity mediated by IgG1 and IgG3 antibodies (Khalife et al., 1989). The mechanism of cytotoxicity mediated by IgE antibodies has been recently reviewed (Capron et al., 1989). More recently, it has been suggested that not only IgE but also IgA antibodies could be

involved in ADCC mediated by human eosinophils: cytotoxicity levels are strongly reduced when eosinophils have been incubated by aggregated IgE but also with monomeric, aggregated or secretory IgA (Capron et al., 1988).

Our strategy of immunization against schistosomiasis has been based on the induction of effector mechanisms specially involving IgE antibodies. One of the best candidate for immunization is represented by SM28GST, which is highly immunogenic, inducing a circulating antibody response, as well as highly protective, leading to 60-80% protection in rodents and primates (Capron et al., 1987a). In this context, it was interesting to investigate the effector mechanisms induced by immunization with SM28GST in rats. First, using an ELISA assay against SM28GST as antigen, it was shown that immunization of rats, in the presence of either aluminium hydroxyde or BCG as adjuvant, induced a strong IgG and IgE antibody response, specially after two injections. Very surprisingly, an IgA antibody response was also induced by such immunization procedure. The induction of high levels of cytotoxicity against schistosomula targets by sera from rats immunized with SM28GST, in the presence of rat eosinophils, clearly indicated that an ADCC mechanism was induced by immunization. The role of IgE antibodies in these immunized rat sera was suggested by heat inactivation, whereas the role of IgA was indicated, by depletion experiments of IgA on anti-IgA immunosorbent. The participation of these two isotypes in eosinophil-dependent cytotoxicity was further confirmed by competition experiments with myeloma IgE or myeloma IgA respectively. Taken all together, these results indicated that immunization with SM28GST induces potent eosinophil-mediated cytotoxicity mechanisms directed against schistosomula, involving not only IgE but also IgA antibodies. The respective contribution of IgE versus IgA, which might play a synergistic role, is presently under investigation.

Eosinophils are not only involved in the destruction of schistosomula targets. Original studies had clearly demonstrated that eosinophils from immune mice, bearing cytophilic antibodies could be incriminated in the destruction of schistosome eggs *in vitro* (James & Colley, 1976) indicating therefore that the effector function of eosinophils could be directed against different stages of the schistosome cycle. The main disease manifestation of schistosomiasis is the granuloma formation. These granulomas are composed of large variety of cell populations including eosinophils. Elegant immunofluorescent studies have shown deposits of one granule protein (MBP) around the schistosome egg, suggesting that eosinophils have degranulated and released their granule contents (Kephart et al., 1988). Moreover, deposits of granule proteins are also found in the surrounding tissues, indicating that these highly toxic molecules could be also involved in tissue damage (Kephart et al., 1988). Not only granule proteins, but also pro-inflammatory mediators such as substance P have been found in granulomas during murine schistosomiasis mansoni (Weinstock et al., 1988). These last two studies, well illustrate the functional duality of eosinophils, playing a part not only in a protective immune response but also in pathology.

The relevance of the *in vitro* effector mechanisms with a protective immune response in vivo has been well established in rats. Either passive transfer of cytotoxic antibody isotypes or adoptive transfer of immune eosinophils in situ, led to the protection of naive rats against a challenge infection (Capron et al., 1982). The *in vivo* correlates of *in vitro* findings, are more difficult to establish in the human situation, than in experimental models. However, the stricking association of eosinophil-dependent cytotoxic antibody isotypes with resistance to reinfection whereas *in vitro* blocking isotypes were mainly detected in susceptible subjects, strongly suggest a participation of eosinophils in antibody-dependent protective response during human schistosomiasis. In particular, it could be first shown that specific IgM antibodies directed against a carbohydrate epitope of one glycoprotein (GP38) and exerting a blocking effect *in vitro,* were in fact detected at significantly higher levels in the case of susceptible children from Kenya, than in the resistant children (Khalife et al., 1986). In a more recent study, performed in the Gambia, it was shown, very interestingly, that specific IgE antibodies increased with age and were correlated with a protective response, whereas IgG4 antibodies (blocking isotype *in vitro*) were decreasing with age and therefore appeared more correlated with susceptibility to reinfection (Hagan et al., 1991). In parallel, in the kenyan study, it could be shown that IgG3 antibodies increase with the age-dependent acquired immunity, similarly to IgA antibodies directed against SM28GST. These 2

antibody isotypes were potent inducers of eosinophil mediated cytotoxicity.

In conclusion, these studies of effector mechanisms during schistosomiasis have clearly indicated that antibodies represented a prominent component of immune response both in rats and in humans. They have also pointed out the importance of an isotypic selection with the existence of effector or blocking antibody isotypes, indicating probably the major role played by cytokines in the regulation of the protective response. Although no direct evidence for the protective role of eosinophils were brought in humans, indirect arguments have clearly suggested a correlation between *in vitro* findings and resistance or susceptibility to reinfection, conforting therefore the strategy of immunization based on the induction of such effector mechanisms. Finally, studies performed in schistosomiasis have brought numerous informations on the various facets of eosinophil functions and led to a better knowledge of eosinophil physiopathology, applicable to other diseases involving eosinophils and specially allergic diseases.

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