

DUAL FUNCTION OF EOSINOPHILS IN PATHOGENESIS AND PROTECTIVE IMMUNITY AGAINST PARASITES

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The functional duality of eosinophils, involved in a protective response or in pathogenesis is illustrated in various parasitic infections. In schistosomiasis, eosinophils have been shown to mediate schistosomula killing, in the presence of antibodies. The association of eosinophil-dependent cytotoxic antibody isotypes with resistance of reinfection (IgE and IgA antibodies), whereas in vitro blocking antibody isotypes (IgG4, IgM) were detected in susceptible subjects, suggested a participation of eosinophils in antibody-dependent protective response. However eosinophils could participate to granuloma formation and consequently to the pathological reactions during schistosomiasis. Activation of eosinophils by antibodies, leading to release of granule proteins have been studied in patients with filariasis. Eosinophil peroxidase, EPO was released after IgE-dependent activation whereas Eosinophil Cationic Protein, ECP, was released after IgG- and IgA-dependent activation of eosinophils, results suggesting a process of differential release of mediators. Interactions between eosinophils and interleukins, and specially IL-5 are discussed. Whereas a receptor for IL-5 has been characterized on human eosinophils, recent studies have shown that eosinophils expressed the messenger RNA encoding IL-5. These results associated to data showing the synthesis of other cytokines indicate that eosinophils are not only the source of cytotoxic mediators involved in the effector phase of immunity but also of growth and regulatory factors, participating to immunoregulation.

Key words: eosinophils – schistosomiasis – protection – cytotoxicity – IL-5

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 γ receptor (Fc γ RII/CD32), Fc ϵ receptor (Fc ϵ RII/CD23) and Fc α receptor (Fc α 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 α (CD11a), P150-95 α (CD11c), the common

P95 β 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 crystalloid 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 (Vaso-

Intestinal Peptide). Very interestingly, it has been discovered that eosinophils not only produced cytotoxic or inflammatory mediators but also cytokines, such as IL-1, TGF- α , 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. The functional duality of eosinophils in protective immunity and pathogenesis can be clearly illustrated in schistosomiasis but also in other parasitic infections. The differentiation, activation and effector functions of eosinophils are under the control of several cytokines, and growth factors. Among them, Interleukin-5 (IL-5) exerts pleiotropic effects on eosinophils. Recent data concerning the characterization of specific IL-5 receptors and the synthesis of IL-5 by eosinophils will be summarized.

EFFECTOR FUNCTION OF EOSINOPHILS IN SCHISTOSOMIASIS

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 adherence 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 Fc ϵ R2 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., 1987).

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 α 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., 1991). 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., 1986b). 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 ag-

gregated IgE but also with monomeric, aggregated or secretory IgA (Capron et al., 1988).

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). These 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 striking 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., 1986b). 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 SM28 GST. These 2 antibody isotypes were potent inducers of eosinophil mediated cytotoxicity.

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, conforing therefore the strategy of immunization based on the induction of such effector mechanisms.

EFFECTOR FUNCTION OF EOSINOPHILS IN OTHER PARASITIC INFECTIONS

Filariasis is often characterized by elevated and persistent eosinophilia with increased antibody levels and in some occasions associated with eosinophil-linked tissue damage (such as endomyocardial fibrosis in *Loa loa*). The interactions between eosinophils and antibodies leading to degranulation and release of granule proteins have been studied in the case of patients with filariasis (Khalife et al., 1986a). In this model, the results suggested a mechanism of differential release of eosinophil mediators. Eosinophil peroxidase, EPO was released only after IgE-dependent activation whereas Eosinophil Cationic Protein, ECP, was released after IgG- and IgA-dependent activation of eosinophils. These findings suggest that mediators released by eosinophils in parasitic infections associated or not with increased IgE levels might differ. They also indicate in relation to tissue localization of eosinophils and local synthesis of IgE and IgA, that eosinophil mediators locally released could be different. Ultrastructural analysis and immunogold labelling suggest in addition that granule pro-

teins were not released by extrusion of whole granules but rather by a process called "piece-meal degranulation" or vesicular transport mechanism (Capron et al., 1989).

Eosinophils have been also associated to the pathogenesis of eosinophil meningitis due to *Angiostrongylus cantonensis* infection (Yoshimura et al., 1988; Perez et al., 1989). Eosinophils might also exert their effector functions in protozoan infections, as shown by the recent studies of Molina & Kierzenbaum (1989a). In summary, deposits of toxic eosinophil granule proteins have been found on heart myofibers during acute Chagas disease, whereas interaction of eosinophils with *T. cruzi* *in vitro* induces bystander cardiac cell damage (Molina & Kierzenbaum, 1989b). These results point to eosinophils as possibly involved in the pathogenesis of Chagas' disease. Eosinophil granule components, which are toxic for *T. cruzi* (Molina et al., 1988) might also play a role in the production of necrotic lesions, well illustrating the functional duality of eosinophils.

IL-5 AND EOSINOPHILS

Regarding the factors responsible for eosinophil differentiation and activation, Interleukin-5 (IL-5) appears to specifically promote the proliferation and terminal differentiation of eosinophil precursors, as well as the prolonged survival of eosinophils *in vitro* (Yamaguchi et al., 1988a, b). It is also a potent activator of eosinophil functions such as cytotoxicity or mediator release. The presence of hypodense eosinophils in hypereosinophilic situations can therefore be related to eosinophils in a state of activation, in addition to increased eosinophilopoiesis. IL-5 might represent the major cytokine involved in the induction of hypodense eosinophils and therefore in eosinophil-mediated pathology (Owen et al., 1989). Similarly to other lymphokines, the biological effects of IL-5 are probably linked to the interaction with specific receptors on susceptible targets. Indeed, the existence of specific receptors for IL-5 has been recently demonstrated on human eosinophils, by binding assays (Chihara et al., 1990). More recently, a molecular approach has led to the cloning of the human high affinity IL-5 receptor, composed of 2 chains α and β (Tavernier et al., 1991). The possible functions of IL-5 R, specially the soluble form of IL-5 R α , will be discussed, in relation to the recently described expression of IL-5 mRNA by human eosinophils (Desreumaux et al., 1992).

Characterization of a receptor for IL-5 on human eosinophils – Binding experiments using ^{125}I -labeled recombinant human IL-5 were performed, in order to demonstrate the existence and to examine the level of expression of a receptor for IL-5 on human eosinophils (Chihara et al., 1990). Radiolabelled IL-5 specifically bound to eosinophils and reached saturation at an approximate concentration of 1 - 1.25 nM. Very interestingly, the levels of specific binding were significantly higher on hypodense blood and tissue eosinophils than on normodense cells. Specific IL-5 R was not detected on human neutrophils, a finding that correlates with the lack of effect of IL-5 on the neutrophil myeloid series. Scatchard analysis revealed that the association constant was five fold greater in the case of hypodense eosinophils than with normodense eosinophils, a result that suggests the previous *in vivo* activation of hypodense eosinophils. However, the number of receptors per cell was not different between the two subpopulations. Moreover, these data indicated that the binding of IL-5 was to a single class affinity receptor, in contrast to results obtained on murine B cells (Mita et al., 1988).

To determine whether the binding of IL-5 to human eosinophils was specific, competition experiment with other lymphokines showing aminoacid sequence homology with IL-5 were performed. Only unlabeled IL-5 but not IL-3, GM-CSF or IFN γ inhibited the binding of radiolabeled IL-5, suggesting the specificity of the binding site for IL-5 on blood eosinophils from patients (Chihara et al., 1990).

It has to be noticed that the existence of such receptors for IL-5 has been further confirmed on human eosinophils in two independent studies (Migita et al., 1991; Ingley & Young, 1991). Taken all together, these results confirm the existence of a high affinity binding site for IL-5 on human eosinophils and eosinophilic subclones of HL60, but not other cell types, including B lymphoma cells, results in agreement with the specific effect of IL-5 on the human eosinophil lineage.

Molecular basis of a high affinity human IL-5 receptor – The detection of IL-5 binding with a high affinity on eosinophilic subclones of HL-60, led Tavernier et al. to initiate a cloning strategy of the human IL-5 Receptor (Tavernier et al., 1991). As a source of mRNA they have used butyrate-induced eosinophilic

subclones of HL-60. A cDNA library was constructed in λ gt11 and screened either with oligonucleotide probes of the murine IL-5 α chain or with a mouse IL-3 R like cDNA, identical to the mIL-5R β chain.

First using degenerated oligonucleotides based on mIL-5R α chain, 41 positive clones were isolated and one was selected for further analysis. Some particular features of the sequence were characteristics of the cytokine and cytokine receptor family. The open reading frame defined a polypeptide which is 71% homologous to the murine IL-5 R α chain, indicating that hIL-5R α belonged to the cytokine receptor gene family. Moreover, the human counterpart is also characterized by the presence of a signal peptide which indicates that the hu IL-5 R α chain can be secreted. The MW calculated from the sequence is 35.9 kD which is far away from the 60 kD MW estimated from cross-linking studies. This is probably due to the existence of six potential glycosylation sites and therefore to the glycosylation of the molecule. On the other hand, the human IL-5R β chain was shown to be identical to the IL-3 R and GM-CSF R β chains (Tavernier et al., 1991).

Northern blot analysis using the cDNA of the hu IL-5 R α chain revealed the presence of a major transcript of 1.4 kb in eosinophilic subclones of HL-60, as well as in human cord blood cells differentiated *in vitro* in eosinophils by culture with IL-5. A chimeric protein was constructed by fusion between the secreted hIL-5R α and the C terminal domain of the anchored mIL-5R α . When this IL-5R α construct was transfected in COS cells, only low affinity binding was observed. Cotransfection with this IL-5 R α and with and IL-5 R β construct led to a 4 fold increased binding affinity, indicating that both chains are required to form the high affinity receptor (Tavernier et al., 1991).

The functions of the soluble h IL-5R α chain have been explored, using the supernatants of COS cells transfected with the IL-5 R α chain. The supernatants, containing soluble IL-5 R α chain could inhibit the binding of radiolabelled m IL-5 to transfected COS cells. In addition, these supernatants also inhibit, in a dose dependent manner, the IL-5 driven eosinophil differentiation from cord blood cells, indicating the antagonistic properties of the hu IL-5 R α chain. The functions of this soluble

IL-5 R α chain *in vivo* have still to be explored. In particular, it would be interesting to evaluate its regulatory role on eosinophil recruitment and activation. The expression of IL-5 R α chain during eosinophil maturation/differentiation, as well as by hypodense versus normodense eosinophils during diseases have to be investigated, as well as the tissue specificity of expression.

IL-5 mRNA expression by human eosinophils – IL-5, the major factor involved in eosinophil differentiation is classically produced by T cells. It has been reported that IL-5 mRNA could be detected by *in situ* hybridization in skin and mucosal bronchial biopsies of patients with eosinophil infiltration (Kay et al., 1991, Hamid et al., 1991) but without a precise identification of the labeled cells. It has been also established that IL-5 mRNA expression can be detected in other cell populations like mast cells (Plaut et al., 1989) or Reed Sternberg cell (Samoszuck and Nansen, 1990). In a very recent study (Desreumaux et al., 1992) we demonstrate the presence of IL-5 mRNA in activated eosinophils. First, eosinophils infiltrating the mucosa of four patients with active coeliac disease strongly expressed the IL-5 mRNA, by *in situ* hybridization. In contrast, no positive signal was obtained in the cell infiltrate from patients submitted to gluten restriction or in the normal duodenum tissues. These results associated to electron microscopy analysis or immunostaining with the EG2 mAb indicate that activated eosinophils present in coeliac disease could synthesize IL-5. Not only tissue infiltrating eosinophils but highly purified eosinophils from the peripheral blood of patients with eosinophilia could express IL-5 mRNA (Desreumaux et al., 1992).

Together, these results suggest that eosinophils have the capacity to synthesize IL-5, which could contribute to paracrine interactions with T and B cells and, in autocrine fashion, could locally participate, through binding to IL-5 receptor, to eosinophil differentiation and activation. The secretion by eosinophils of both IL-5 and antagonistic soluble IL-5 R α chain might constitute the basis of a subtle T cell-independent regulation of eosinophil differentiation and activation and should be explored in parasitic diseases. In addition to their inflammatory and effector functions, eosinophils may thus serve as a source of growth and regulatory factors with a broad range of biological effects. Although both di-

rect and indirect arguments favouring the effector function of eosinophils have been obtained in parasitic diseases, several questions remain unsolved, specially concerning eosinophilopoiesis and eosinophil activation. The recent advances obtained in the field of interleukins would certainly contribute, in the near future, to a better understanding of eosinophil functions.

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