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Invited Review

Mechanisms underlying eosinophil trafficking and their relevance *in vivo*

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Summary. After their formation in the bone marrow, eosinophils circulate with a short half-life and are distributed throughout the body, especially in mucosal and sub-mucosal regions. Although a small amount of these cells are normally seen in healthy tissue, blood and tissue eosinophilia is a hallmark of helminthic and allergic diseases. The role of eosinophils in the normal physiology of mucosal tissues is not understood, but there is good evidence to demonstrate that these cells protect the host at least against some intestinal helminths, specially those with a lung cycle. In addition, there are now many data that support a role for eosinophils in the pathophysiology of allergic diseases, such as asthma. Because helminthic diseases have been largely controlled in developed countries, there has been much interest in the development of drugs which affect eosinophil migration and/or activation in the tissue and which may, thus, be useful in the treatment of allergic conditions. The understanding of the mechanisms controlling eosinophil trafficking and/or activation are essential in the development of anti-eosinophil-based therapeutic strategies. The present paper reviews aspects of eosinophil biology with emphasis on the role of eosinophils in parasitic infections and allergy, the basic mechanisms underlying the trafficking of eosinophils into tissue and how these can be modulated pharmacologically.

Key words: Eosinophil, Allergy, Helminths, Trafficking, Interleukin-5, Cell adhesion molecules

Eosinophil morphology and granules

Eosinophils are a type of granulocyte derived from the bone marrow and distinguished by their morphological features, constituents, products, and association with specific diseases. These cells are present in blood and characteristically in tissues with an epithelial interface with the environment, such as the respiratory, gastrointestinal, and lower genitourinary tracts (Weller, 1991). In blood, eosinophils account for only 1 to 3 percent of peripheral leukocytes in healthy subjects, and the upper limit of the normal range is 350 cells/ml of blood. The normal numbers of eosinophils in tissues is not known, but there are normally many more cells in tissues than in blood (Weller, 1991). In addition, as we will discuss below, eosinophil numbers in blood and tissue increase several times in the presence of certain conditions, such as asthma and helminth infection.

Typically, human eosinophils measure 8 pm in diameter, have a bilobed nucleus and contain characteristic granules that are known to have an intense avidity for eosin dye (Hirsch and Hirsch, 1980). Three types of granules are present in their cytoplasm: (i) primary granules, which are round, uniformly electron dense, and characteristically present in eosinophilic promyelocytes; (ii) specific or secondary granules, which are composed of an electron-dense core and an electron-lucent matrix; and (iii) small granules (Dvorak et al., 1988, 1991). Other cytoplasmic structures of the eosinophil are lipid bodies, which are non-membrane-bound, lipid-rich inclusions found in many types of cells (Dvorak et al., 1983). The latter structure appears to be particularly important for the production of lipid mediators by activated eosinophils (Bozza et al., 1997).

Eosinophils can express receptors for several molecules, including immunoglobulins (IgG, IgE, IgA), complement (C1q, C3b/C4b, iC3b, C5a), cytokines (IL-3, IL-5, GM-CSF), chemokines (eotaxin, eotaxin-2, RANTES, MCP-3, MCP-4), lipid mediators (PAF, LTB_4) and steroids (estrogens, glucocorticoids). It is via these receptors that eosinophils receive messages from and respond to their environment. Thus, the importance of each of these receptors will be dictated according to the environment in which the cells are situated. In addition to the receptors cited above, eosinophils express a range of cluster-determinant (CD) antigens on their surface. Although the function of several CD antigens is

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not known, their position on the plasma membrane suggests they may play an important role in cell/cell contact and communication. Among the CD antigens expressed in eosinophils, there are several cell adhesion molecules (CAMs) - CD11/CD18, VLA-4 (α4ß1), α4ß7 and CD62L (L-selectin).

Secretory products and cytokines

The ability of eosinophils to cause damage to cells or tissues appears to be related to their great ability to secrete a range of substances which include cationic proteins (see Table 1), cytokines, chemokines, lipid mediators and oxygen-derived radicals (Table 2). The action of each individual substance is beyond the scope of this review, but the wide array of substances produced and secreted gives a good indication of the ability of eosinophils to affect different cells or systems (see Giembycz and Lindsay, 1999 for review). Although the cationic proteins are usually thought to be specifically found in eosinophils, basophils have been shown to contain about one fourth as much MPB as eosinophils, and to contain detectable amounts of EDN, ECP and EPO, although at levels lesser than 7% of those in eosinophils (Abu-Ghazaleh et al., 1992). In addition, small amounts of EDN and ECP are also present in neutrophils and could be synthesized by these cells (Abu-Ghazaleh et al., 1992).

Table 1. Cationic proteins and other enzymes present in eosinophil granules

CATIONIC PROTEIN	MAIN ACTIVITY DESCRIBED
MBP (14 KD) major basic protein	- toxic to helminthic and protozoan parasites, tumor cells, bacteria, host cells - causes bronchospasm - induces histamine release - degranulates basophils - increases the expression of IL-8 mRNA
EPO (15-55 KD) Eosinophil peroxidase	- toxic to helminthic and protozoan parasites, bacteria, tumor cells and host cells. - induces histamine release - inactivates the peptide-leukotrienes - converts LTC_4 to all-trans isomers of LTB_4
ECP (18-21 KD) Eosinophil cationic protein	- toxic to helminthic and protozoan parasites, bacteria and host cells. - promotes degranulation of mast cells - neurotoxin
EDN (18 KD) Eosinophil-derived neurotoxin	- neurotoxin

Birth, life and death (IL-5)

Eosinophils are produced in bone marrow from pluripotential stem cells. The latter differentiate first into hybrid precursors with properties of basophils and eosinophils and then into a separate eosinophil lineage (Boyce at al., 1995). Three cytokines – interleukin-3 (IL-3), interleukin-5 (IL-5) and granulocyte-macrophage colony stimulating factor (GM-CSF) – have an essential role in the production of eosinophils. Interestingly, these cytokines are encoded by closely linked genes on chromosome 5q31 and bind to receptors that have a common beta chain and different alpha chains. Interleukin-5 (also known as eosinophil-differentiation factor) is the most specific colony factor for the eosinophil lineage and is responsible for selective differentiation of eosinophils (Sanderson, 1992). The critical role of IL-5 in the production of eosinophils is best demonstrated by genetic manipulation in mice. Overproduction of IL-5 in transgenic mice results in profound eosinophilia (Sanderson, 1992), and deletion of the IL-5 gene is accompanied by a marked reduction of eosinophils in the blood and tissue of naive animals or of sensitized animals after an allergen challenge (Foster et al., 1996; Kopf et al., 1996). In addition to a critical role for IL-5, much recent interest has been placed in the understanding of the role chemokine eotaxin and the eotaxin receptor (CCR3) play in the production and release of eosinophils from the bone marrow. It has been recently demonstrated that eotaxin alone or acting synergistically with IL-5 stimulates the release of eosinophils from bone marrow into the peripheral circulation (Collins at al., 1995). In addition, eotaxin was effective as GM-CSF at inducing eosinophil differentiation both *in vivo* and *in vitro* in mice (Peled et al., 1998). Thus, eotaxin induces not only the release of eosinophils from bone marrow, but also the generation of leukocytes needed for an inflammatory reaction. This is in line with findings that eotaxin knock-out mice had considerably lower eosinophil counts than control littermates in one study (Rothenberg et al., 1997). However, the latter findings have been recently disputed when the eotaxin gene was knocked out in mice from another genetic background (C57Bl6 x 129/Sv) (Yang et al., 1998).

In blood, eosinophils circulate with a half-life of approximately 18 hours (Spry, 1993). However, in

Table 2. Eosinophil-derived substances

CLASS OF PRODUCTS	CYTOKINES	CHEMOKINES	LIPID MEDIATORS	NEUROPEPTIDES	OXYGEN-DERIVED RADICALS
Products known to be secreted	GM-CSF, IL-3, IL-5, IL-1 α, IL-2, IL-4, IL-6, IL-10, IL-16, TGF-ß, TNFα, TGF-α	RANTES, eotaxin, MIP-1α, IL-8, IP-10, MIG	PGE_2, PGD_2, $PGF_{2\alpha}$, Thromboxane, LTC_4, PAF	SP, VIP, CGRP	O_2^-, H_2O_2, OH^-, NO, singlet oxygen

tissues eosinophils may survive for weeks (Spry, 1988), depending on cell matrix interactions and the cytokines present in the microenvironment (Rothenberg et al., 1987). For example, the VLA-4-dependent interaction of eosinophils with fibronectin induces the autocrine secretion of GMC-SF by eosinophils leading to their increased survival *in vitro* (Anwar et al., 1993).

Like other cells, eosinophils can be triggered to undergo programmed cell death or apoptosis, a process accompanied by condensation of the cytoplasm, segmentation of the nucleus and extensive degradation of chromosomal DNA, via specific surface death receptors (Simon and Alam, 1999). One of these death receptors expressed by eosinophils is CD95 (Fas/APO-1) (Matsumoto et al., 1995; Tsuyuki et al., 1995; Druilhe et al., 1996; Hebestreit et al., 1996). The ligand of CD95 (CD95L, FasL, APO-1L) is highly expressed by activated T cells (Green and Ware, 1997). The activation of CD95 leads to stimulation of a protease cascade, which, when started, is irreversible (Nagata, 1997). These proteases belong to the IL-1-converting enzyme (ICE) family of cysteine proteases, now called caspases (Alnemri et al., 1996), and appear to be directly responsible for the induction of apoptosis. There is evidence that at least two members of this family, caspase 3 and caspase 8, are involved in the regulation of eosinophil apoptosis (Simon and Alam, 1999). Inhibition of eosinophil apoptosis can be achieved by at least two mechanisms: (1) increased expression of eosinophil survival factors, and (2) disruption of death signals (Simon and Alam, 1999). *In vitro*, eosinophil survival can be maintained by the eosinophil haematopoietins, IL-3, IL-5 and GM-CSF (Her et al., 1991; Yamaguchi et al., 1991), that stimulate an anti-apoptotic signalling pathway via the common ß chain of their receptors (Simon and Alam, 1999). Stimulation of eosinophils with IL-5 or other hematopoietins results in tyrosine phosphorylation of kinases Lyn (Pazdrak et al., 1995a; Yousefi et al., 1996), Jak1 (Ogata et al., 1998) and Jak2 (Pazdrak et al., 1995b; van der Bruggen et al., 1995; Simon et al., 1997), that activate the Stat family of nuclear factors. Indeed, IL-5 activates Stat 1 (Pazdrak et al., 1995b; van der Bruggen et al., 1995), Stat 3 (Caldenhoven et al., 1995) and Stat5 (Mui et al., 1995) nuclear factors. This pathway can be blocked by TGF-ß, for example, that inhibits the tyrosine phosphorylation of Jak2 and Lyn tyrosine kinases (Pazdrak et al., 1995a). Besides the cytokines, nitric oxide can also disrupt the apoptotic signaling pathways initiated via CD95 (Hebestreit et al., 1998).

The role of eosinophil apoptosis in the control of eosinophil accumulation in tissue is not entirely understood. Theoretically, induction of eosinophil apoptosis in tissue would resolve tissue eosinophilia without activation of local inflammatory responses. However, to date this hypothesis has only been shown convincingly in the study by Tsuyuki and colleagues (1995) who showed that local treatment of mice with anti-CD95 resolved the lung eosinophilia following challenge of sensitised mice with ovalbumin.

Eosinophil trafficking - Cell adhesion molecules

In response to an appropriate stimulus, circulating leukocytes interact with endothelial cells prior to leaving blood vessels and entering the tissue. The current model for the accumulation of leukocytes into tissues of the systemic circulation predicts that there are at least three stages of leukocyte/endothelial cell interaction (Carlos and Harlan, 1994; Springer, 1994; Teixeira et al., 1995). Initially, circulating leukocytes are captured and roll on the endothelial cells of post-capillary venules. The rolling leukocyte may then be activated by chemoattractants (e.g. eotaxin, interleukin-8, LTB4) and this leads to upregulation and increased avidity of integrins present on the surface of the leukocyte (e.g. CD11/CD18 and VLA-4). Integrins mediate the firm adhesion of activated leukocytes to endothelial cells by binding to ligands including ICAM-1 and VCAM-1. The leukocytes are then able to migrate to the interstitium, a process that also involves adhesion molecules, including the integrins and PECAM-1 (CD31), present at intercellular junctions. Here, the intention is not to review the basic cell adhesion pathways, but to highlight studies evaluating the effects of cell adhesion-based strategies on eosinophil recruitment *in vivo*.

There are several differences in the adhesion pathways utilised by eosinophils which are specific for these cells in relation to other leukocytes, specially neutrophils (Teixeira et al., 1995). These differences could help in the development of eosinophil-specific therapies without unwarranted side-effects on the host defence properties of other cell types (Teixeira et al., 1995). Of the known cell adhesion molecules much interest has been placed in understanding the role of selectins and the integrins CD18 and VLA-4 in mediating eosinophil recruitment *in vivo* (Lobb and Hemler, 1994; Teixeira et al., 1995) (see Table 3).

The first step in the adhesion cascade is the loose interaction between the circulating eosinophil and endothelial cells. This process is named rolling and is mediated by the selectin family of adhesion molecules present on eosinophils (L-selectin) or endothelial cells (P- and E-selectin) and their carbohydrate-expressing ligands (eg. PSGL-1) (Carlos and Harlan, 1994; Varki, 1994). The exact interplay between these molecules is not fully understood, although recent observations suggest that neutrophil rolling can be mediated by P-selectin and L-selectin sequentially (reviewed by Ley and Tedder, 1995; Kansas, 1996). Moreover, it is clear from studies assessing neutrophil migration *in vivo* that the function of selectins is partially redundant (Bosse and Vestweber, 1994; Labow et al., 1994; Henriques et al., 1996) and that the integrins VLA-4 and α4ß7 may also play a role in mediating the rolling of alpha4 integrin-positive cells *in vitro* and *in vivo* (Sriramarao et al., 1994; Alon et al., 1995; Kanwar et al, 1997). A role for selectins in mediating eosinophil recruitment *in vivo*

Table 3. Studies evaluating the effects of anti-cell adhesion molecules (CAMs) on eosinophil migration *in vivo*.

CAM TARGETED	SRATEGY	SPECIES	EFFECTS OBSERVED	REFERENCE
Adhesion molecules present on endothelial cells:				
P-selectin/ICAM-1/VCAM-1 Antibodies (Abs)	knock-outs (KO),	mice	Inhibition of rolling in P-selectin KO and inhibition of recruitment to the peritoneal cavity in P-selectin and ICAM-1 KO in the presence of anti-VCAM-1 antibodies.	Broide et al, 1998a
P-selectin/ICAM-1	KO	mice	Inhibition of eosinophil recruitment in the lung of ICAM-1 and P-selectin KO. Loss of inhibition in P-selectin KO with time.	Broide et al., 1998b
P-/E-selectin/CD11b/CD18	Abs	mice	Inhibition of eotaxin-induced eosinophil recruitment.	Das et al., 1997
P-selectin	KO	mice	Inhibition of eosinophil recruitment in the lung and inhibition of AHR	De Sanctis et al., 1997
P-/E-/L-selectins	Abs	mice	Inhibition of LPS-induced pleural eosinophilia; P- and E-selectins are functionally redundant.	Henriques et al., 1996
P/E-selectins/VLA-4	Abs	mice	Inhibition of eosinophil recruitment in skin sites;reliance on selectins depends on whether a direct-acting chemoattractant or an allergic reaction are studied.	Teixeira and Hellewell, 1998
ICAM-1	Ab	monkey	Blockade of tissue eosinophilia and AHR.	Wegner et al., 1990
ICAM-1	Ab	monkey	In animals with chronic AHR and airway eosinophilia, anti-ICAM-1 does not prevent disease but prevents recurrence after treatment with steroids.	Gundel et al., 1992
ICAM-1	KO	mice	Inhibition of airway hyperresponsiveness (AHR), lung eosinophilia.	Wolyniec et al., 1998
ICAM-1	Ab	rat	Inhibition of antigen-induced airway eosinophilia.	Chin et al., 1998
ICAM-1/ CD11/CD18	Ab	rat	Inhibition of allergen-induced nasal eosinophilia, antibodies given during immunisation.	Asakura et al., 1996
ICAM-1	Ab	rat	Inhibition of antigen-induced airway eosinophilia.	Richards et al., 1996
ICAM-1	Abs	rat	Inhibition of AHR but no effect on airway eosinophilia after antigen.	Sun et al., 1994
ICAM-2	KO	mice	Delayed eosinophil migration but prolonged accumulation, heightened AHR.	Gerwin et al., 1999
ICAM-1/VCAM-1	KO	mice	Both inhibit eotaxin-induced transmigration, but VCAM-1 appears more relevant later.	Jia et al., 1999
VCAM-1/ICAM-1	KO	mice	Inhibition of pulmonary eosinophilia.	Gonzalo et al., 1996
VCAM-1	Ab	mice	Inhibition of cutaneous eosinophilia in contact hypersensitivity.	Satoh et al., 1997
VCAM-1/VLA-4/ICAM-1	Ab	rat	Inhibition of IL-4-induced cutaneous eosinophilia, no effect of anti-ICAM-1.	Sanz et al., 1998
VCAM-1/VLA-4	Ab	rat	Inhibition of TNFα-induced cutaneous eosinophilia.	Sanz et al., 1997
Adhesion molecules present on leukocytes:				
L-selectin/VLA-4	Abs	guinea pig	Inhibition of eosinophil recruitment, AHR and M2 receptor dysfunction with anti-VLA-4, but not anti-L-selectin.	Fryer et al., 1997
L/P-Selectins	Fucoidin	guinea pig	The selectin-binding polysaccharide fucoidin blocks eosinophil migration by blocking both P- and E-selectin function.	Teixeira and Hellewel, 1997
CD11/CD18	Ab	guinea pig	Inhibition of eosinophil migration in skin.	Teixeira et al., 1994b
CD11/CD18	Abs	guinea pig	Inhibition of eosinophil numbers in BAL, but not tissue, inhibit AHR (one Ab).	Milne and Piper, 1994
CD11/CD18	Ab	guinea pig	Blockade of tissue eosinophilia and AHR.	Noonan et al., 1991
CD11/CD18	Abs	guinea pig	Blocks eosinophil migration in naive and primed skin sites.	Macari et al., 1996
CD11/CD18/VLA-4	Abs	guinea pig	Anti-CD18 blocks eosinophil migration in naive and primed sites. Anti-VLA-4 blocks responses induced by TNFα only.	Macari et al., 1998
CD11/CD18/VLA-4	Abs	guinea pig	Either treatment alone inhibits BAL, but not tissue, following sephadex. Combined treatment affects tissue eosinophilia.	Das et al., 1995
CD11/CD18/VLA-4	Abs	rat	Both antibodies block AHR. Airway eosinophilia is blocked by anti-CD11b, but not anti-VLA-4.	Laberge et al., 1995
CD11/CD18/VLA-4	Abs	rat	Inhibition of 5-oxo-ETE-induced lung eosinophilia by anti-CD11a and anti-VLA-4, not anti-CD11b.	Stamation et al., 1998
CD11/CD/18/VLA-4	Abs	rat	Partial inhibition of BAL and parenchymal lung eosinophilia by either anti-CD18 or anti-VLA-4. Combined treatment abrogates eosinophilia	Schneider et al., 1999
CD11/CD18/VLA-4	Abs	mice	Inhibition of eotaxin-induced lung eosinophilia and and BHR in IL-5 transgenic mice by anti-VLA-4 but not anti-CD11b; synergism when both used together.	Hisada et al., 1999
VLA-4	Ab	guinea pig	Inhibition of lung and BAL eosinophilia, no effect on AHR.	Milne and Piper, 1995
VLA-4	Ab	guinea pig	Inhibition of airway eosinophilia and AHR after antigen challenge.	Sagara et al., 1997
VLA-4	Ab	rat	Inhibition of airway eosinophilia and AHR after antigen challenge.	Richards et al., 1996
VLA-4	Ab	guinea pig	Inhibition of airway eosinophilia, free EPO and AHR after antigen challenge.	Kranevel et al., 1997
VLA-4	Ab	guinea pig	Inhibition of nasal eosinophilia and eosinophil activation.	Terada et al., 1996
VLA-4	Ab	guinea pig	Inhibition of airway eosinophilia and AHR after antigen challenge.	Pretolani de al., 1994
VLA-4	Abs	sheep	Blockade of AHR, late phase reaction with little effect on eosinophil influx.	Abraham et al., 1994
VLA-4	Ab	guinea pig	Blockade of eosinophil recruitment in skin.	Weg et al., 1993
VLA-4/VCAM-1/ICAM-1 CD11/CD18	Abs	mice	Anti-VLA or anti-VCAM-1, but not anti-CD11b or anti-ICAM-1, block eosinophil recruitment in the trachea of antigen-challenged mice.	Nakajima et al., 1994

was initially demonstrated in a murine model of lipopolysaccharide (LPS)-induced pleural eosinophilia (Henriques et al., 1996). In this model, blocking antibodies against L-selectin or a combination of anti-P- and anti-E-selectin monoclonal antibodies (mAbs) virtually abolished LPS-induced eosinophil recruitment. In addition, several studies with knock-out mice have confirmed the importance of the selectins, specially the endothelial selectins (P and E) in mediating eosinophil rolling and recruitment *in vivo* (see Table 3). In these experiments however, it has been difficult to evaluate whether the antibodies or the knocked-out gene inhibited eosinophil recruitment directly or indirectly by modulating the recruitment and/or activation of lymphocytes and macrophages. More recently, we have shown that the combined treatment with anti-selectin antibodies abolished eosinophil recruitment induced by direct-acting chemoattractants or in allergic reactions in murine skin (Teixeira and Hellewell, 1998). The latter studies clearly demonstrate that selectin-based therapies do have a direct effect on eosinophil trafficking *in vivo*. Moreover, it was clear from these studies that eosinophil recruitment in response to direct acting mediators was mostly P-selectin-dependent, whereas that in a delayed-onset allergic reaction it was dependent on both P- and E-selectin (Teixeira and Hellewell, 1998). These findings highlight the redundant function of P- and E-selectin at mediating both neutrophil and eosinophil recruitment in models of chronic inflammation. Moreover, they suggest that blockade of both endothelial selectins is necessary if effective inhibition of leukocyte recruitment at sites of chronic inflammation is to be achieved pharmacologically. Interestingly, in a delayed-type hypersensitivity (DTH) reaction, eosinophil recruitment was independent of P-, E- or L-selectin (Teixeira and Hellewell, 1998). The inability of anti-selectin antibodies to block eosinophil recruitment in the DTH reaction suggests there are additional selectin-independent adhesion pathways which may mediate eosinophil rolling in these late phase skin reactions. Two possibilities include a role for VLA-4 in mediating eosinophil rolling in conditions of chronic inflammation mimicked by the DTH reaction or the recently described cell adhesion molecule which plays an important role in mediating bovine lymphocyte rolling *in vitro* (Jutila et al., 1997). The ability of anti-VLA-4 antibodies to block eosinophil recruitment in sites of DTH reactions support the former hypothesis, but definite proof that anti-VLA-4 blocks rolling and not firm adhesion (see below) in the model is still lacking (Teixeira and Hellewell, 1998). Nevertheless it is worth noting that studies have demonstrated that anti-VLA-4 blocked the rolling of eosinophils *in vivo* (eg. Sriramarao et al., 1994).

Studies with human eosinophils highlight differences in the ability of neutrophils and eosinophils to use differentially P- and E-selectin for rolling *in vitro*. This is in line with the idea that the selectin ligand(s) on eosinophils is different from that of neutrophils (Symon et al., 1996). If such a concept is true, it would then be possible to devise eosinophil-selective drugs which would block their migration, but not those of neutrophils, *in vivo*. The inhibition of eosinophil migration by selectin-active drugs (eg. fucoidin) has been demonstrated *in vivo* (Teixeira and Hellewell, 1997), but no studies have proved the concept that a differential action on eosinophils versus neutrophils is possible *in vivo*.

The interaction selectin/selectin ligand approximates eosinophils to endothelial cells and allows these cells to roll on the endothelial surface. The rolling leukocyte is activated by molecules acting on 7-transmembrane receptors and this activation leads to an increase in the function of integrins on the eosinophil surface. Proof that activation of such receptors is relevant for eosinophil migration *in vivo* has been recently provided using pertussis toxin which binds to and inactivates certain G proteins coupled to 7-transmembrane receptors (Teixeira et al., 1997a). Several studies have evaluated the ability of anti-integrin antibodies on the recruitment of eosinophils *in vivo* (see Table 3). Although anti-CD18- or anti-ICAM-1-based strategies are very effective at suppressing eosinophil recruitment to a range of different tissues, these strategies also interfere with the ability of neutrophils to migrate into sites of tissue inflammation (Springer, 1994, Henriques et al., 1996). Thus, specificity would be lost and most interest is now centred on the role of VLA-4 or its ligands (especially VCAM-1) in mediating eosinophil recruitment *in vivo*.

VLA-4 integrin is constitutively expressed on the surface of eosinophils, but not neutrophils (with the exception of rat neutrophils), and appears to play an important role in the binding of eosinophils to cytokine-activated endothelial cells *in vitro* (Teixeira et al., 1995).

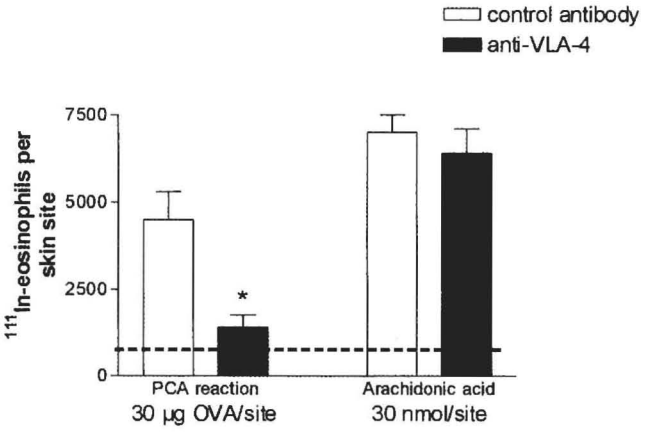

Fig. 1. Effect of an anti-VLA-4 monoclonal antibody (2B4) on the migration of eosinophils induced by arachidonic acid or in a passive cutaneous anaphylactic (PCA) reaction in guinea pig skin. Animals were injected i.v. with the monoclonal antibody (2B4, 3mg/kg) and antigen (ovalbumin, OVA) or arachidonic acid injected intradermally. ^{111}In-eosinophils (3 x 10^6/animal) were then administered i.v. and their accumulation in the skin sites assessed over a 2-hour period. Results are the mean ± s.e. mean of 5 animals. *: when $P < 0.05$ when compared to animals injected with control antibody (MOPC21).

Table 4. Examples of chemoattractant molecules known to induce eosinophil migration *in vivo*.

Lipid mediators: PAF, LTB4, 5-HETE
Complement fragments: C5a, C3a
Chemokines: eotaxin, RANTES, MCP-3, eotaxin-2, MCP-4, MIP-1a, IL-8
Cytokines[1]: IL-2, IL-5, TNFα, IL-16
Components of microorganisms: FMLP, lipopolysaccharide[1]
Others[2]: histamine, ECF-a, PGD2

[1]: usually an indirect effect via the release of chemokines or lipid mediators *in vivo* and chemokinetic *in vitro*; [2]: small activity *in vitro* and very weak activity *in vivo*.

Blockade of VLA-4 is followed by suppression of eosinophil recruitment to sites of inflammation in most, but not all, studies (Table 3). Common to the studies which observed an inhibition was the prolonged time-course required for eosinophil recruitment, usually occurring after or concomitantly with other cell types (e.g. lymphocytes, monocytes, mast cells) which are themselves inhibited by anti-VLA-4 treatment. Using two different anti-α4 mAbs we evaluated the role of α4-dependent pathways on the rapid accumulation of ^{111}In-eosinophils into sites of allergic and non-allergic inflammation. We demonstrated that ^{111}In-eosinophil accumulation induced in a passive allergic reaction, but not after injection of arachidonic acid, was effectively inhibited by pretreatment with anti-α4 antibodies (Fig. 1). The choice of arachidonic acid to compare to the allergic reactions was based on our previous findings that both reactions were dependent on the local release of leukotrienes (Teixeira and Hellewell, 1994). In addition, pretreatment of eosinophils with anti-α4 mAbs prior to their injection *in vivo* was not followed by inhibition of their recruitment. These results suggest that the eosinophil may not be the only target of the inhibitory effects of systemically administered anti-α4 mAbs, specially when rapid accumulation of these cells is studied. Other cell targets include an effect of anti-α4 antibodies on the recruitment of monocytes, lymphocytes and, possibly, basophils, in addition to an effect on the function of mast cells (Carlos and Harlan, 1994; Springer, 1994).

A few studies have evaluated the ability of anti-CD18 and anti-α4 to synergise at inhibiting allergen-induced eosinophil recruitment *in vivo* (see Table 3). In the mouse trachea, an anti-CD18 mAb had no effect on antigen-induced eosinophil infiltration when used alone, but significantly enhanced the inhibitory effects of an anti-α4 mAb (Nakajima et al., 1994). In contrast, in a rat model of lung inflammation, an anti-α4 mAb had no effect on eosinophil influx and did not increase the ability of an anti-CD18 mAb to inhibit bronchoalveolar lavage or tissue eosinophilia (Laberge et al., 1995). In our guinea pig skin model, eosinophil recruitment in response to non-allergic inflammatory stimuli was CD18-dependent and there was no further inhibition when an anti-α4 mAb was used (Teixeira and Hellewell, unpublished). Allergen-induced inflammation was inhibited by anti-CD18 and anti-α4 mAbs and there was no synergy when both mAbs were used. This last piece of evidence suggests that these two adhesion pathways, CD18-dependent and α4-dependent, are positioned in series and do not represent redundancy of the system. One possibility, at least in the guinea pig cutaneous model of inflammation, is that the α4-dependent pathway is important for the adequate release of mediators from mast cells and the CD18 pathway is responsible for the adhesion of eosinophils to endothelial cells.

There are no reports on the ability of anti-integrin-based strategies to modulate eosinophil recruitment in human allergic diseases. One interesting observation is the presence of eosinophils in tissues of patients with leukocyte adhesion deficiency I (LAD I, lack CD18-dependent adhesion) (Springer, 1994). The latter observation suggests that alternative pathways, presumably VLA-4/VCAM-1, mediate the migration of these cells. Studies in human diseases are needed to understand the efficacy and specificity (side-effects) of the blockade of anti-VLA-4/VCAM-1 versus anti-CD18/ICAM-1 as modulators of eosinophil recruitment.

Eosinophil trafficking - chemoattractant molecules

The activation of eosinophils by chemoattractant molecules which act on 7-transmembrane receptors is a critical step in the adhesion cascade (Teixeira et al., 1997a). The activation of such receptors induces an elevation of intracellular calcium in eosinophils which precedes their migration *in vivo* (Teixeira et al., 1997a). Thus, it appears that molecules acting on serpentine receptors are capable of inducing eosinophil migration *in vivo* by directly activating the eosinophil. These molecules include lipid mediators (e.g. PAF and LTB4), complement fragments (e.g. C5a) and chemokines (e.g. eotaxin) (see Table 4). In addition, several mediators which act on receptors distinct from the serpentine receptors, including the cytokines IL-5, TNFα and IL-2, have been shown to induce the chemokinesis of eosinophils *in vitro* and their accumulation in tissues (Weller, 1991; Milne et al., 1995; Giembycz and Lindsay, 1999). For example, TNFα is chemokinetic for eosinophils *in vitro* and its *in vivo* administration induces the accumulation of eosinophils in the skin of guinea pigs (Macari et al., 1998 and references therein). However, TNFα appears to act indirectly via the production of a yet unidentified chemoattractant molecule (Macari et al., 1998). Similarly, the effects of IL-2 and IL-5 on the accumulation of eosinophils *in vivo* is not demonstrated in all studies (e.g. Milne et al., 1995) and appears to be indirect, possibly dependent on the release of chemokines such as eotaxin.

Recently, there has been much interest in a group of chemoattractants, the chemokines, which appear to play a major role in mediating eosinophil recruitment *in vivo*. Chemokines are a group of chemoattractant proteins

with molecular weight usually ranging from 8 to 12 kDa and aminoacid sequence identity of 20 and 90% (Power and Wells, 1996). These proteins possess 4 conserved cysteine residues as part of their structure and, depending on the presence or absence of one aminoacid between the first two cysteines adjacent to the N terminus, are classified in C-C or C-X-C chemokines. There are two other additions to the family – a C chemokine (lymphotactin) which possesses only two cysteine residue and a C-X_3-C chemokine (fractkaline) which possesses three intervening aminoacids (see Baggiolini, 1998 for review). Chemokines are produced in both acute and chronic inflammatory reactions, but also appear to control leukocyte trafficking under basal conditions (Matthews et al., 1998). The great interest in chemokines stems from their ability to selectively activate and recruit particular leukocyte subsets. This selectivity is based on the differential expression of chemokine receptors among different leukocyte subsets (Murphy, 1996; Baggiolini, 1998). For example, human eosinophils express high levels of the chemokine receptor CCR3 (40,000 to 400,000 receptors/eosinophil) and low levels of the CCR1 receptor (1 to 5% of the CCR3 levels) (Daugherty et al., 1996; Ponath et al., 1996). Thus, chemokines which activate the CCR3 receptor (e.g. eotaxin, RANTES, MCP-3) are capable of activating several eosinophil functions *in vitro*. The importance of the CCR1 receptor for the activation of human eosinophils is a matter of controversy, but, at least in some allergic individuals, chemokines which activate this receptor (e.g. MIP-1α) do activate the function of eosinophils.

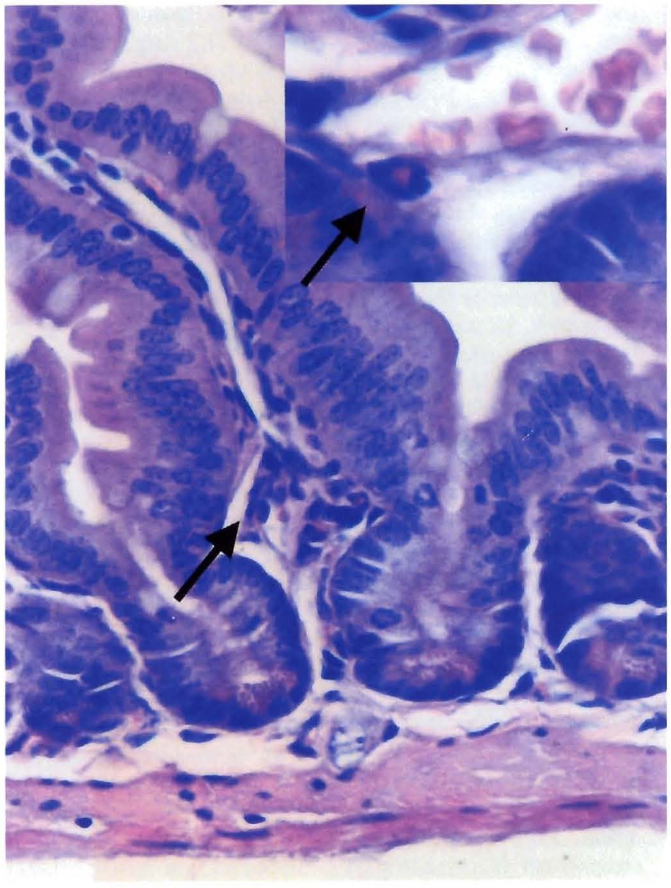

Fig. 2. Histological aspect of the intestinal mucosa of a germ-free mouse. (x 400) Note the marked decrease in the number of cells infiltrating the submucosa of these animals. A few eosinophils are also seen (arrow). In the insert, an eosinophil is seen leaving a blood vessel (x 1000)

In a murine model, we have recently shown that the intradermal injection of the C-C chemokines eotaxin and MIP-1α, but not RANTES or MCP-5, induced the recruitment of eosinophils to skin sites (Teixeira et al., 1997b; Teixeira, 1998). Interestingly, an anti-eotaxin antibody, but not an anti-MIP-1α antibody, inhibited the recruitment of eosinophils to sites of active anaphylactic reactions in mouse skin (Teixeira et al., 1997b). Together these data demonstrate the effectiveness of eotaxin on inducing eosinophil migration *in vivo* and the importance of endogenous eotaxin for the migration of eosinophils to sites of allergic reactions. This is in agreement with recent findings of defective eosinophil migration to sites of pulmonary anaphylaxis in eotaxin knock-out mice (Rothenberg et al., 1997). However, even in the knock-out animals, there was marked eosinophil migration in the later stages following antigen challenge and, in one study, no inhibition of eosinophil migration was observed (Rothenberg et al., 1997; Yang et al., 1998). These studies highlight an important aspect not to be missed when evaluating the effects of chemokines – there is much redundancy in the system and inhibition of one chemokine may be overcome by some other chemokine with a similar functional profile. In the case of eosinophils, blockade of eotaxin may be insufficient for inhibiting eosinophil recruitment since other chemokines, such as RANTES, MCP-3 and eotaxin-2, may replace eotaxin functionally. As eosinophils have one dominant chemokine receptor, one possibility to overcome such a problem would be the development of CCR3 receptor antagonists. At least one study has shown the feasibility of this approach. Pre-treatment of animals with the CCR3 receptor antagonist MetRANTES effectively inhibited the recruitment of eosinophils in anaphylactic reactions in mouse skin (Teixeira et al., 1997b).

What is the role of the eosinophil?

The functional importance of eosinophils in immunology is usually considered under patho-physiological conditions. In other words, studies which evaluate the role of eosinophils are usually carried out in disease states, such as asthma or parasitic infections. This is most likely a problem of immunology itself which tends to evaluate the role of cells, immuno-globulins or reactions in the light of defence against invading organisms, but not in the light of a normally

functioning organism. Thus, there remains an important question to be evaluated: what role do eosinophils play under normal conditions, i.e. what is the physiological role of eosinophis? As mentioned above, eosinophils are typically seen in mucosal tissues and are even found in the intestinal mucosa of germ-free animals (Fig. 2). It would be logical then to associate the physiological function of these cells with the normal function of mucosal tissues, which itself is difficult to define. As mucosal tissues are an important interface between the organism and its environment and, if we believe these are essential places for the entrance of antigens, eosinophils are at an important interface to function as antigen-presenting cells (reviewed by Weller and Lim, 1997). In this way, eosinophils would deal not only with antigens from invading microorganisms, but also, and more importantly, with the great majority of antigens we meet everyday - those which are ingested or inhaled. The understanding of this physiological role of eosinophils is far from clear but it may shed light on the understanding of the role these cells play in human disease. Below, we will consider the role eosinophils play in helminthic infections and allergic diseases.

Eosinophils and helminth infections

Helminth infections are highly prevalent in human populations, particularly in tropical and subtropical countries. Twenty-six species of helminth parasites have been reported to infect humans. The four most prevalent species of nematodes - *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale* and *Trichuris trichiura* - infect almost three billion people worldwide (Warren et al., 1990; Bundy, 1994).

The three hallmarks of gastrointestinal (GI) infection are eosinophilia, intestinal mastocytosis and IgE production (Love et al., 1976; Ruitenberg et al., 1979; Jarrett and Miller, 1982). These three elements are also associated with the immune response during allergic disease, like asthma (see below). The IgE production, eosinophilia and mastocytosis are regulated by cytokines produced predominantly by a T helper cell subset in human and mice designated Th-2 (Mossmann and Coffman, 1989; Romagnani, 1994). The Th-2 subset produces predominantly IL-4, IL-5, IL-9, IL-10 and IL-13; these cytokines regulate the humoral response, promoting B cell proliferation and immunoglobulin switching to non-complement-fixing IgG isotypes, IgG1 in mice or IgG4 in human, and to IgE-producing plasma cells (reviewed by Abbas et al., 1996; Mosmann and Sad, 1996). Moreover, Th-2 cytokines, especially IL-5, induce eosinophil proliferation, differentiation and survival (see above). Although the CD4+ T cell separation into Th-1 and Th-2 has been very well demonstrated *in vitro* and associated, *in vivo*, with the outcome of some infections, such as Leishmania major infection in mice, it appears that such division is an oversimplification of the immune response. For example, the measurement of cytokines in the thoracic duct lymph of Trichinella spiralis-infected rats demonstrated that, even though the intestinal immune response has a predominant Th-2 profile with high levels of IL-5 and IL-4, IFN-γ was also present (Ramaswamy et al., 1996).

Recent evidence favours the idea that a protective role exists for Th2-like responses in helminthic infection (Urban et al., 1992). There are at least two independent studies of murine infection with *Trichuris muris* (Else et al., 1992) and *Heligmosomoides polygyrus* (Urban et al., 1991) that have directly demonstrated a protective role of the Th2 response. In *T. muris* infection, the mouse strains that predominantly produce Th-1 responses develop a chronic infection and are defined as susceptible strains for this parasite. The mouse strains capable of mounting a Th2 response against *T. muris* infection are able to eliminate worms before they mature into egg-producing adult worms, and are designated resistant strains. Susceptible mouse strains are able to cure the chronic infection produced by *T. muris* if they are treated with IL-4 (Else et al., 1994).

The mechanism by which IL-4 acts to control a helminth infection is still unknown. IL-4 is essential for the induction of IgE responses (Finkelman et al., 1988) and, together with the IL-4-induced Th2 response, is also necessary for the intestinal mastocytosis, at least in mice (Madden et al., 1991). Also, IL-4 production is normally associated with increase in IL-5 and, consequently, eosinophilia. Therefore, any or all of these three hallmarks of helminthic infection – eosinophilia, IgE and mastocitosis - might participate in the protection. We will review and discuss the association of IgE, mast cell and eosinophils with helminthic infections and the possible role of these immune mechanisms in protective immunity. However, it is important to underline that the importance of the IL-4-dependent response observed during helminthic infection is not fully understood. Urban et al. (1995) have recently suggested that IL-4 may have a direct effect on the parasite and/or the intestinal mucosa that might contribute to worm elimination. *In vitro*, prolonged stimulation with IL-4 stimulation produced a dose-dependent proliferation of a rat intestinal epithelial cell line - IEC-6 (McGee and Vitkus, 1996). Colgan et al. (1994) also showed that IL-4 stimulation of the T84 cell line (a human gut epithelium cell line) resulted in a modulation of the barrier function and ion transport displayed by the monolayer. In the same system, IL-4 regulated the expression of accessory molecules that mediate neutrophil adhesion on the monolayer. Finally, Ramaswamy et al. (1994) reported an IgE transport mechanism in the intestine of *T. spiralis*-infected rats, which is also IL-4 dependent. Thus, IL-4 may have a host protective role against intestinal helminths by a mechanism not entirely dependent on its ability to modulate IgE or mast cell activities.

The participation of IgE in protective immunity against helminthic infections is still unclear. However, several studies have reported a correlation between

specific IgE levels and protection against infection with different species of helminths - *Taenia taeniformis* (Musoke et al., 1978), *Trichinella spiralis* (Dessein et al., 1981; Negrão-Correa et al., 1999), *Brugia malayi* (Kurniawan et al., 1993), Strongyloides ratti (Korenaga et al., 1986), *Necator americanus* (Pritchard et al., 1995) and *Schistosoma ssp* (Dunne et al., 1992; Hagan, 1993). A more direct evidence for IgE involvement in protective immunity has been achieved by transfer of parasite-specific IgE in two infection models in rats. In *S. mansoni* infection, a purified IgE-monoclonal antibody specific for adult worm metabolic antigen passively transferred a significant level of protection to adoptive recipients (Verwaerde et al., 1987). In *T. spiralis*-infected mice the transfer of immune IgE to intestinal primed rats will lead to rapid expulsion of the parasite (Ahmad et al., 1991). Moreover, IgE immunoprecipitated from 14 day-*T. spiralis*-infected rats was able to abolish the ability of adult worm to invade IEC-6 (rat epithelial cell line) monolayer (Negrão-Corrêa, 1997).

Due to the low concentration of IgE in serum, its function is normally associated with the receptor(s) to which IgE binds on cells. Therefore, two mechanisms are postulated to explain the protective role of IgE against helminthic infection: immediate hypersentivity and antibody-dependent cellular cytotoxicity (ADCC) reaction. The high affinity receptor for IgE, FcεRI, is expressed in high density by basophils and mast cells. Immediate hypersensitivity, the principal known function of IgE *in vivo*, requires binding of IgE to FcεRI receptors on mast cells or basophils. The cross-linking of IgE on these cells by antigen triggers the release of preformed mediators, such as histamine, heparin, proteases and cytokines and the synthesis and secretion of newly formed lipid-derived mediators and cytokines (Schwartz, 1994). The release and action of these mediators induce the characteristic symptoms observed in an allergic reaction and contribute to the chronic inflammation that can appear in the surrounding tissue. The FcεRI receptor has also been detected on dendritic cells (Grabbe et al., 1993) and on human eosinophils purified from hypereosinophilic pacients (Gounni et al., 1994). The function of IgE on either of these cell types is still unknown. In human eosinophils, IgE binding to FcεRI can lead to an ADCC reaction which has been associated with killing of schistosomula of *Schistosoma mansoni in vitro* (Gounni et al., 1994). However, murine eosinophils do not express the high (or low) affinity IgE receptor (de Andres et al., 1997).

IgE also has a low affinity receptor, FcεRII (CD23), present on a variety of cell types, including platelets, lymphocytes, enterocytes and eosinophils (Capron and Joseph, 1991). IgE also binds to epsilon-binding proteins (εBP) detected on the surface of enterocytes (Brassart et al., 1992), eosinophils and neutrophils (Truong et al., 1993a,b), mast cells and macrophages (Frigeri and Liu, 1992). Although binding of IgE to these cells and triggering with specific antigen may mediate ADDC killing of parasite larvae or other cellular functions *in vitro*, IgE-dependent cell functions *in vivo* for most of these cell types have not been unequivocally established.

The participation of mast cells in the protective mechanisms against GI infection was initially suggested based on the temporal coincidence of intestinal mastocytosis and worm expulsion. The theoretical mechanism, known as "Allergic Inflammation" (Larsh and Race, 1975), suggested that the local inflammatory reaction stimulated by mast cell degranulation led to worm elimination by non-specific mechanisms. The release of mast cell mediators in the intestinal mucosa may induce changes in mucosal permeability (Murray, 1972), plasma leakage and further infiltration of leukocytes (Askenase, 1979). Large amounts of histamine could also increase the production and release of mucus from goblet cells (Lake et al., 1980) and increase intestinal motility (Goldhill et al., 1997). All these alterations were thought to make the intestinal mucosa an unsuitable environment for the parasite. The idea was reinforced with the demonstration that *T. spiralis* worms transplanted into a normal rat intestine were able to survive for many days while the worms transplanted into inflamed intestine were eliminated rapidly (Wakelin and Wilson, 1979). Indication of a protective role for mast cell against intestinal helminths has also been demonstrated in mast cell-deficient mice (W/W^v mice). W/W^v mice showed a more severe *T. spiralis* (Alizadeh and Murrell, 1984), *S. ratti* (Nawa et al., 1985) and *Hyminolepis nana* (Watanabe et al., 1994) infection, with a slower worm expulsion than control mice. The direct connection between the response in W/W^v mice with mast cell function exclusively should be considered more carefully. The mutation observed in W/W^v mice affects a tyrosine kinase (c-kit) receptor for stem cell factor (SCF) that is also involved in the growth and differentiation of erythroid and granulocyte precursors, as well as lymphoid stem cells (Witte, 1990).

Mast cell degranulation cannot entirely explain the participation of specific IgE in protective mechanisms operating against all helminthic infections, since the course of *N. brasiliensis* infection in W/W^v mice was unchanged (Uber et al., 1980). Morever, in *T. spiralis* infection, the transfer of immune IgE plus immune CD4+ OX22- T cells resulted in rapid expulsion of the infective larvae from the rat intestine (Ahmad et al 1991). However the protective T cell population (CD4+ OX22- T cells) induced an intestinal eosinocytosis, but not an intestinal mastocytosis (Wang et al., 1990). Furthermore, during worm elimination, our preliminary work using an *in vitro* system indicated that intestinal-specific IgE might participate directly in protective mechanism independently of mast cells (Negrão-Corrêa, 1997). Thus, although mast cells may play a role in protection against some helminthic infections and mast cell-derived products affect parasite survival in some systems, direct evidence for a role for mast cells has not been convincingly obtained for all the helminthic models *in vivo*.

Table 5. The effects of IL-5 on helminthic infections: IL-5 neutralization by treatment with anti-IL-5 or anti IL-5 receptor (IL-5R) antibodies.

PARASITE	EFFECT	REFERENCE
Schistosoma mansoni	After vaccination with irradiated cercariae, anti IL-5-treated and untreated mice eliminated similarly the challenge infection.	Sher et al. 1990
Schistosoma japonicum	Anti IL-5-treated and untreated mice showed similar egg elimination, although the granuloma volume was reduced in anti IL-5-treated mice.	Cheever et al., 1991
Trichinella spiralis	Anti IL-5-treated and untreated mice had similar muscle larvae burden after the primary and the secondary infection.	Herdon and Kayes, 1992
Strongyloides venezuelensis	During the primay infection, the anti IL-5 plus anti IL-5R treatment did not alter the egg production or adult worm elimination time. However, the number of adult worms in intestine was significantly higher in challenged infected IL-5-depleted mice.	Korenaga et al., 1991
Strongyloides venezuelensis	During primary infection, anti IL-5-treated mice had significantly higher number of intestinal adult worms than untreated mice	Korenaga et al., 1994
Angiostrongylus cantonensis	Anti IL-5-treated mice showed more intracraneal worms than untreated mice.	Sasaki et al., 1993
Onchocerca volvulus	Vaccination with irradiated L3 larvae was capable of killing larvae implanted in untreated mice but not anti IL-5- or anti IL-4-treated mice	Lange et al., 1994

In the same way, the role of eosinophils in protective mechanisms against helminth have been very contradictory. As mentioned above, in most helminth-infected hosts, like *S. mansoni*-infected mice (Sher et al., 1990), *Protostrongylus rufescens* and *Haemonchus contortus*-infected lambs (Mansfield and Gamble, 1995), *Paragonimus westermani* and lymphatic filariosis in humans (Kan et al., 1995; Magnussen et al., 1995) *Trichinella spiralis*- and *Nippostrongylus brasiliensis*-infected rats (Watanabe et al., 1988) and in *Strongyloides ratti*-infected rats (Moqbel, 1980), the number of eosinophils in peripheral blood and tissues involved in parasite migration increases dramatically. The association of eosinophil and protective mechanisms against helminthic infections was proposed after the demonstration of the effectiveness of the depletion of eosinophils by anti-eosinophil antiserum in modulating infection *in vivo*. Thus, in *Trichostrongylus columbriformis*-infected guinea pigs (Gleich et al., 1979), anti-eosinophil antibody treatment was able to increase the host susceptibility to the parasite, and, in *T. spiralis*-infected mice treated with anti-eosinophil antibodies (Grove et al., 1977), the number of muscle larvae recovered was twice as much as in the control infected mice. At the same time as these *in vivo* studies were published, some authors also demonstrated that immune serum plus rat or human eosinophils were capable of mediating killing of *Schistosoma mansoni* schistosomula and *Trichinella spiralis* larvae (Capron et al., 1981; Butterworth, 1984) or *S. hematobium* schistosomula (Hagan et al., 1985) *in vitro*. The cytotoxic effect of eosinophils on antibody-coated helminth larvae has also been demonstrated *in vivo* on *T. spiralis* newborn larvae of infected rats (Wang and Bell, 1992) and on *Onchocerca volvulus* microfilaria *in vitro* (Folkard et al., 1996). Eosinophils were the main effector cells involved in the killing of *S. stercoralis* L3 larvae implanted, inside a chamber, into the peritoneal cavity of immune mice (Abraham et al., 1995). The mechanisms underlying the ability of eosinophils to kill parasite larvae is not entirely understood, but is most likely related to the ability of eosinophils to secrete various cytotoxic proteins and mediators (see Tables 1 and 2), specially the toxic activity of cationic proteins and oxygen-derived radicals.

The regulation of eosinophil production and differentiation in the bone marrow, its migration to the tissue and survival in tissue are effectively and selectively influenced by IL-5 (see above). In this sense, strategies which block the production and/or function of IL-5 are specially important for the understanding of eosinophil function *in vivo*. Pioneering studies by Coffman et al. (1989) showed that the injection of monoclonal antibody to IL-5 suppressed the blood eosinophilia and tissue eosinophil infiltration in mice infected with *Nippostrongylus brasiliensis*. Therefore, depletion of IL-5 by specific antibody treatment or genetic manipulation of the IL-5 gene-disruption of IL-5 in IL-5-deficient mice (Kopf et al., 1996) and increased IL-5 expression in IL-5 transgenic mice (Dent et al., 1990) have been used as models to study the role of eosinophils in the protective mechanisms against helminthic infections. In Tables 5 to 7, we summarize the results obtained with different helminthic infections in these IL-5 manipulated hosts. The most obvious conclusion when analysing data in these tables is that, although all the helminthic infection stimulated an IL5-dependent eosinophilia, IL-5 manipulation did not produce a similar effect on infection outcome of all the species evaluated. Evidence for a protective role of IL-5 has been observed mainly on Nematodes with a pulmonary phase in the life cycle (Tables 5 to 7). As discussed above, diversity in the protective mechanism against different species of helminths has also been observed when evaluating the role of mast cells, goblet cells and IgE. Thus, IL-3 treatment of nude mice restored mucosal mastocytosis and the capacity for elimination of *S. ratti* infections; however, this treatment

Table 6. The effects of IL-5 on helminthic infections: IL-5 transgenic mice.

PARASITE	EFFECT	REFERENCE
Mesocestoides corti	The infection was fatal in IL-5 transgenic and non-transgenic mice.	Strath et al., 1992
Schistosoma mansoni	After vaccination with irradiated cercariae, IL-5 transgenic mice and non-trangenic littermates showed similar resistence to challenge infection.	Freeman Jr et al., 1995
Schistosoma mansoni	After vaccination with irradiated cercariae, IL-5 transgenic mice carried more adult worms of challenge infection than the littermate control.	Dent et al., 1997
Trichinella spiralis	Infection in IL-5 transgenic mice or in the littermate controls showed no difference in the muscle larvae and intestinal adult worm recovery, or in the female fecundity.	Hokibara et al., 1997
Trichinella spiralis	Infection in IL-5 transgenic (low transgenic Tg1) mice resulted in more muscle larvae than in infected non-transgenic control.	Dent et al., 1997
Toxocara canis	After ES antigen immunization, the challenge infection in IL-5 transgenic mouse resulted in similar number of larvae than in non-transgenic controls	Sugane et al., 1996
Nippostrongylus brasiliensis or *Toxocara canis*	The number of tissue worms was similar in *T. canis* infected IL-5 transgenic and non-transgenic littermates. IL-5 transgenic mice had less intestinal *N. brasiliensis* and the females were less fecund than the controls.	Dent et al., 1999
Nippostrongylus brasiliensis	During primary infection, IL-5 transgenic mice showed lower number of lung and intestine worms than the littermate control. Moreover eosinophil transference from the transgenic to naive mice resulted in lower larvae burden in lungs.	Shin et al., 1997
Angiostrongylus cantonensis	IL-5 transgenic mice showed lower numbers of intracraneal worms than non-transgenic littermates. The female worms recovered from IL-5 transgenic mice were smaller than the controls.	Sugaya et al., 1997

was not effective against *N. brasiliensis* infection (Abe et al., 1992; Horii et al., 1993; Ishikawa et al., 1994). Goblet cell hyperplasia, and more precisely, alteration of the terminal sugars of goblet cell mucins during *N. brasiliensis* infection is thought to be a key event leading to the elimination of adult worms (Ishikawa et al., 1993). Recently, we described (Negrão-Corrêa et al., 1996) a powerful intestinal IgE response that was initiated during a *T. spiralis* infection of rats. However, the intestinal IgE response of rats was not a universal consequence of a nematode GI infection. In rats infected with *T. spiralis* and *H. polygyrus*, IgE could be measured in the lumen of the small intestine; however, IgE was not detected in the intestinal lumen of *N. brasiliensis*-infected rats (Negrão-Corrêa et al., 1999). All these results reinforce the idea that, even though the immune response to different nematodes seems similar, the mechanism responsible for worm elimination may be selective and specific for each (or some) nematode species.

Interestingly, although the IL-5 manipulation work has been performed in mice (Tables 5 to 7), most of the *in vitro* protection and killing assays routinely use human or rat eosinophils. In this respect, it is worth remembering that De Andres et al. (1997) were not able to demonstrate expression of IgE receptors on mice eosinophils. The lack of IgE receptors in mice eosinophils should affect the ADCC killing of the parasite, suggesting that mice may not be a good model to evaluate the importance of eosinophils in helminthic infections. Another important consideration is that IL-5-deficient mice have proportionally less B1 cells; a B cell population that expresses CD5 antigen and localize in peritoneal cavity and intestinal mucosa (Murakami and Honjo, 1995; Kopf et al., 1996). A possible role of these cells in protection against helminthic parasites has not been evaluated.

Overall, these infection models do suggest that eosinophils might not be essential for the protective immune response against many helminths in mice. However, little data are available in other species and, specially, in humans. These are essential experiments to be carried out in order to evaluate the impact of eosinophil-based treatment on helminthic infectivity.

Eosinophils and allergic diseases

There is much circumstantial evidence to suggest a role for Th2 lymphocytes and eosinophils in the pathophysiology of allergic diseases, such as rhinitis (Klementsson, 1992), dermatitis (Bruijnzeel-Koomen et al., 1992; Grewe et al., 1998) and conjunctivitis (Foster et al., 1991). In these conditions, eosinophil numbers or eosinophil-derived mediators are elevated in tissue and appear to correlate positively with symptoms and/or loss of organ function. However, the case for a role of eosinophils in the pathophysiology of asthma is much stronger and a considerable amount of work has been published on the subject. Asthma is a common disease worldwide and its prevalence appears to be increasing, specially in developed and urban populations (Lebowitz and Spinaci, 1993). Furthermore, asthma sufferers possess a significant degree of disability, and loss of working-days can lead to important economic losses.

Initial evidence of a possible role for eosinophils in the pathophysiology of asthma derived from studies in peripheral blood demonstrating that eosinophils were primed, i.e. were hypodense or secreted more lipid

Table 7. The effects of IL-5 on helminthic infections: IL-5 or IL-5 receptor alpha chain (IL-5R) deficient mice.

PARASITE	EFFECT	REFERENCE
Hymenolepis diminuta	Infection in IL5 (-/-) mice did not prolong the worm survival or improve the parasite growth .	Ovington and Behn, 1997
Mesocestoides corti	IL5 (-/-)-deficient mice did not alter the infection outcome.	Kopf et al., 1996
Brugia malayi	After immunization with killed microfilariae, the challenge infection in IL5 (-/-) did not produce lung pathology or airway hyperresponsiveness (AHR) to cholinergic agonists as non-deficient mice did. The infection level was not measured by the authors.	Hall et al., 1998
Toxocara canis	Lung pathology due the infection was less intense in IL5 (-/-) mouse, however the larvae number and the location did not alter in IL5 (-/-) compared to the non-deficient control.	Takamoto et al., 1997
Trichinella spiralis	The IL-5 (-/-) mice showed higher worm burden after the challenge infection than the non-deficent littermates.	Revised by Matthaei et al., 1997
Strongyloides ratti	During the primary infection, IL5 (-/-) mice had more intestinal worms and eliminated more eggs/larvae than the non-deficient mice, however the adult elimination timing was similar, and both animals were resistent to secondary infection.	Ovington et al., 1998
Angiostrongylus cantonensis	IL5 R (-/-) mice showed an enhanced survival of worms than the controls.	Yoshida et al., 1996
Angiostrongylus cantonensis	IL5 R (-/-) mice yielded a higher number of worms at 20 dpi than the non-deficient littermates.	Sugaya et al., 1997
Heligmosomoides polygyrus	IL 5 (-/-) mice showed higher parasite burden than the controls.	Revised by Matthaei et al., 1997

mediators or enzymes when activated (Smith, 1992). More recently, studies have used invasive techniques to evaluate the lung (e.g. bronchoscopy) and these techniques have been instrumental in the understanding of the pathological and immunological alterations present in the asthmatic lung (Djukanovic et al., 1990). Thus, bronchoscopic studies assessing bronchoalveolar lavage and biopsies of the lungs of asthmatic individuals under basal conditions and following antigen challenge have demonstrated that: (i) airway inflammation is central to the pathophysiology of asthma and appears to play an important role in the development of airway hyperresponsiveness to non-specific stimuli causing bronchoconstriction (reviewed by Djukanovic et al., 1990; Bousquet et al., 1992); (ii) the infiltration of eosinophils in the mucosa, a characteristic feature of the inflammation in the asthmatic lung, is present even in patients with newly diagnosed asthma (e.g. Laitinen et al., 1993); (iii) the numbers and/or activation state of eosinophils or the levels of eosinophil-derived secretory products correlate positively with bronchial hyperresponsiveness (e.g. Wardlaw et al., 1988; Djukanovic et al., 1990); (iv) there is an increase in the levels of eosinophils following antigen challenge and this appears to correlate positively with the ensuing late phase response (e.g. Dupuis et al., 1992; O'Byrne et al, 1987); (v) the eosinophilic lung inflammation is maintained by cytokines predominantly of the TH2 phenotype (Robinson et al., 1992); (vi) these TH2 cytokines are secreted mostly by activated CD4+ T cells (Ying et al., 1993, Walker et al., 1994), but also by mast cells (Bradding et al., 1994); and (vii) treatment with steroids effectively inhibits lung inflammation and that this appears to be the mechanism underlying the effects of these drugs in asthma (Barnes, 1998a). Thus, the current hypothesis is that asthma is a T lymphocyte-driven inflammatory disease of the airways where an increased number of activated eosinophils appear to act as major effector cells. Very importantly, although the clinical studies demonstrate the correlation between eosinophil numbers or activation state with asthma severity, no studies have proved that the correlation is indeed causal. The lack of proof derives from the lack of pharmacological strategies to inhibit specifically the function and/or activation state of eosinophils in human diseases.

Overall, animal studies demonstrate that there is a good correlation between the development of airway hyperresponsiveness (AHR) following antigen challenge and influx of activated eosinophils in the lung (Teixeira et al., 1995). In agreement with this finding, specific blockade of eosinophil migration with antibodies against IL-5 blocks the development of AHR in several species (Reviewed by Teixeira et al., 1995). For example, pretreatment of guinea pigs with an anti-IL-5 antibody (TRFK-5) abrogated both the recruitment of eosinophils and the development of AHR following antigen challenge (eg. Chand et al., 1992; Mauser et al., 1993). Similar results have been obtained in rats, mice and primates (Kung et al., 1995, Mauser et al., 1995, Egan et al., 1997; Yagi et al., 1997). Significantly, pulmonary eosinophilia, AHR and lung damage are abolished in sensitised and challenged IL-5-deficient mice (Foster et al., 1996). However, reconstitution of IL-5 with recombinant vaccinia viruses completely restored aeroallergen-induced eosinophilia and airway dysfunction (Foster et al., 1996). As seen in Table 3, inhibition of eosinophil migration with anti-CAMs is also commonly associated with inhibition of AHR.

One alternative approach to evaluate the role of

eosinophils in the development of AHR is to induce an accumulation of eosinophils in the lung with various strategies and then evaluate lung function. For example, we have recently shown that the intratracheal injection of the chemokine eotaxin into the lungs of IL-5-transgenic mice is associated with a significant increase in eosinophil numbers in the airways, free EPO levels in BAL fluid and AHR (Hisada et al., 1999). However, there are several studies which demonstrate that the simple presence of eosinophils in the lung is not sufficient to induce AHR, these cells must be activated (Pretolani et al., 1994). In this regard, Lefort et al. (1996) have recently shown that an antibody against MBP effectively blocked the AHR following antigen challenge in guinea pigs.

Despite a great deal of work demonstrating the importance of airway eosinophilia to the development of AHR, there are several studies which convincingly dissociate airway eosinophilia and AHR (reviewed by Smith, 1992; Morley, 1993). For example, the pretreatment of guinea pigs with anti-VLA-4 antibody reduced airway eosinophilia but had no effect on AHR in one study (Milne and Piper, 1995). Similarly, there was no correlation between the presence of airway eosinophilia and AHR in different strains of ovalbumin-sensitised and challenged mice (Wilder et al., 1999). In addition, it is also important to reiterate that animal models of allergic asthma only mirror part of the pathophysiology of asthma and not the disease as seen in man. For example, the changes in AHR seen in sensitised/challenged animals when compared to naive animals is orders of magnitude smaller than those observed in asthmatic versus normal individuals (Smith, 1992). So, what can we learn from animal models and how can we extrapolate it to the human diseases? Possibly, the most important message from studies in animal models is that eosinophils do appear to play an important role in the pathophysiology of AHR, but there are certainly other factors which contribute to the modification of pulmonary function. Moreover, it appears that specific inhibition of eosinophil recruitment and/or function is an important strategy to be pursued when developing new anti-asthma drugs (Teixeira et al., 1995). However, definite proof of a role of eosinophils in the pathophysiology of human asthma is still to be shown.

Pharmacological modulation of eosinophil trafficking

Thus, if eosinophils are leukocytes with an important effector function in allergic diseases, it is possible that drugs which modulate eosinophil recruitment and/or activation may be useful therapeutic agents for these diseases. In addition, novel therapeutic strategies may arise from the understanding of the mechanisms underlying eosinophil recruitment and/or activation. The central point in this working hypothesis is the central role of eosinophils in the pathophysiology of allergic conditions, such as asthma. As mentioned above, the evidence towards such an unique role is very strong but definite proof is still lacking. Thus, it is not known whether specific inhibition of eosinophil recruitment and/or action will result in improvement of asthma. In addition, one central assumption in the use of anti-eosinophil-based strategies is that these will be used in patients (from developed countries) in whom the risk of helminthic infection is low.

Glucocorticosteroids are the "gold standard" for the treatment of eosinophilic conditions in man and against which novel drugs and therapies must be compared. Indeed, steroids are probably the most effective inhibitors of the recruitment of eosinophils in allergic models of inflammation in animals and possibly the only class of drugs which have been successfully used against eosinophilic diseases in man (Teixeira et al., 1995; Barnes, 1998a). At the molecular level, steroids bind to specific receptors in the cytoplasm and that leads to the translocation of the complex to the nucleus of the cell where it will inhibit or induce the synthesis of a great range of proteins (Barnes, 1998b). For example, steroids block the synthesis of the Th2 cytokines IL-4 and IL-5 and this inhibition may account for some of the inhibitory effects of steroids on eosinophil recruitment observed *in vivo*. Steroids may also induce the synthesis of a group of proteins named lipocortins which have been shown to modulate the recruitment of leukocytes (Das et al., 1997 and references therein). Nevertheless, in one study, blockade of lipocortin had no effect on the inhibitory actions of dexamethasone in mice (Teixeira et al., 1998). Finally, a direct effect of steroids on eosinophils appears to play a role in the modulation of the recruitment of these cells *in vivo* (Teixeira and Hellewel, 1996). Despite their efficacy, the use of high doses of steroids is accompanied by a series of important side effects, such as metabolic disturbances and decreased resistance to infections. Thus, a new "anti-eosinophil" drug should ideally be as effective as or more effective than the steroids but possess fewer side-effects. So far, no such drug exists but there are a few potential candidates. The possible use of anti-CAMs as therapeutic agents is discussed above. Although most experiments to date have used antibodies, there are a several companies developing small molecules with anti-CAM activity which could be administered orally. The availability of these molecules for testing in different animal models and clinical trials is eagerly awaited. Nevertheless, inasmuch as strategies which block the function of CAMs do not appear to be eosinophil-specific, a range of side effects related to inhibition of other leukocytes (e.g. increased susceptibility to infection) may also occur in the presence of such drugs.

The development of drugs which block the action of chemoattractant mediators is probably the strategy that has deserved the greatest attention throughout the years (Teixeira et al., 1995). The availability of a defined receptor with a defined binding molecule facilitates the development of antagonists, specially with molecular biology techniques and robotics that allow large-scale

screening. There are good antagonists that block PAF and leukotriene B_4 receptors and there has been much recent interest in the development of chemokine receptor antagonists, specially antagonists for the CCR3 receptor. Although this strategy achieves a great degree of specificity and, presumably, fewer side-effects, there is much concern about the efficacy of using antagonists at specific receptors. In both acute and chronic inflammatory reactions, there is much mediator redundancy. In other words, there could be several eosinophil chemoattractants released at the site of inflammation and the inhibition of only one may not be sufficient for inhibition of eosinophil recruitment. Nevertheless, these questions are only theoretical and clinical trials must definitely be carried out to refute or confirm the effectiveness of any mediator receptor antagonist. To use an example from another system: although there are many factors which control blood pressure, treatment with angiotensin receptor antagonists is an effective means of treating hypertension. A few inflammatory mediator receptor antagonists have been tested in clinical trials of human asthma. Although preclinical studies, specially in guinea pigs, suggested an important role for PAF receptor antagonists in the treatment of asthma, most clinical studies have failed to demonstrate any significant effect of PAF receptor antagonists in clinical asthma (see for example Kuitert et al., 1995). In contrast, leukotriene receptor antagonists (e.g. zafirlukast and montelulkst) do provide therapeutic benefit in clinical asthma when given alone or in addition to inhaled steroids (Busse et al., 1999; Kemp et al. 1999; Malmstrom et al., 1999). However, leukotriene receptor antagonists were no better than currently used long-acting ß2-adrenoceptor agonists (salmeterol, Busse et al., 1999) or inhaled steroids (budesonide, Malmstrom et al., 1999) for asthma control.

One other alternative to controlling eosinophil influx is via the use of drugs, called functional antagonists, which block the recruitment of these cells whatever the stimuli or mediators involved. The glucocorticosteroids are an example of functional antagonists. More recently, much interest has been placed on drugs which elevate cyclic AMP as modulators of eosinophil recruitment *in vivo* (Teixeira et al., 1997c). It is hoped that these drugs will be as effective as steroids but with fewer side-effects or, at least, side-effects which would be easily reversed upon discontinuation of the drug. The levels of cyclic AMP in a given leukocyte are controlled by a balance between the rate of cyclic AMP production via G protein-coupled adenylate cyclase and the rate of cyclic AMP degradation by phosphodiesterases (PDEs) (Teixeira et al., 1997c). Although agents which elevate cyclic AMP by activating adenylate cyclase possess the ability to block eosinophil migration and/or activation acutely, it appears that most effects of these drugs are lost chronically (Giembycz, 1996). The reasons for such a loss of anti-inflammatory effects is not entirely understood but could be related to the ability of elevated levels of cyclic AMP to induce PDEs in different cell types (Teixeira et al., 1997c; Giembycz and Lindsay, 1999).

The PDE family of enzymes is subdivided into 7 subfamilies based on their biochemical, pharmacological and genetic characteristics. One interesting aspect of the biology of PDEs is that a subfamily of enzymes, namely PDE4, appears to be the major modulator of cyclic AMP levels in cells which participate in the inflammatory process (reviewed in Teixeira et al., 1997c; Giembycz and Lindsay, 1999). As such, PDE4 inhibitors are effective inhibitors not only of the functional response of eosinophils but also of their ability to migrate into sites of inflammation *in vivo* (Teixeira et al., 1994a, 1997d; Giembycz and Lindsay, 1999). In addition to their anti-inflammatory activity, PDE4 inhibitors also possess some bronchodilatory activity which may have an additional usefulness in the treatment of asthma (Giembycz and Dent, 1992). Moreover, PDE4 inhibitors do not appear to lose their anti-inflammatory efficacy after continuous administration *in vivo*. Although no PDE4 inhibitor has been tested successfully in the clinic, clinical usefulness derived from the treatment of asthmatic patients with the non-specific PDE inhibitor theophylline suggests this to be an interesting and promising approach.

The central role of IL-5 in eosinophilopoiesis, maturation, priming and survival in tissue (see above) makes the inhibition of this cytokine one of the most powerful targets to inhibit eosinophil recruitment and activation *in vivo*. However, the development of drugs which bind to and block the IL-5 receptor has proven very difficult and no inhibitor has been reported to date. Nevertheless, there are several antibodies which bind to and inactivate IL-5 function (see above). Results from clinical trials evaluating the use of these antibodies in human disease are eagerly awaited and should strongly make a case for (or against) the efficacy and safety of eosinophil-based therapeutic strategies. Whether the use of antibodies for diseases is feasible in chronic conditions such as asthma is unlikely, but a few studies in animals demonstrate that anti-IL-5 antibodies are effective even 3 to 6 months after their administration (Egan et al., 1997).

Concluding remarks

Much is now known about the mechanisms underlying eosinophil recruitment *in vivo* and the ability of these cells to release several toxic substances in tissues. In addition, much data have been gathered to suggest an important role for eosinophils in the physiopathology of allergic diseases, such as asthma. In the next few years, we will be able to determine whether this suggestion is correct and whether eosinophil-based therapeutic strategies are indeed effective for the treatment of allergic diseases. However, if these strategies are effective and patients are treated in such a way, we will need to very carefully examine their effects on helminthic diseases, specially in developing countries where they are more prevalent. Meanwhile, much research is needed to understand the physiology of eosinophils in the absence of

disease and the role of these cells in helminthic infection models in species other than the mouse.

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