

- 8 Briskin, M.J. *et al.* (1993) MAdCAM-1 has homology to immunoglobulin and mucin-like adhesion receptors and to IgA1. *Nature* 363, 461–464
- 9 Wright, P.E. and Dyson, H.J. (1999) Intrinsically unstructured proteins: re-assessing the protein structure–function paradigm. *J. Mol. Biol.* 293, 321–331
- 10 Dawkins, R. *et al.* (1999) Genomics of the major histocompatibility complex: haplotypes, duplication, retroviruses and disease. *Immunol. Rev.* 167, 275–304
- 11 Murphy, P.M. (1993) Molecular mimicry and the generation of host defense protein diversity. *Cell* 72, 823–826
- 12 Agrawal, A. *et al.* (1998) Transposition mediated by *RAG1* and *RAG2* and its implications for the evolution of the immune system. *Nature* 394, 744–751
- 13 Dias Neto, E. *et al.* (2000) Shotgun sequencing of the human transcriptome with ORF expressed sequence tags. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3491–3496
- 14 Smith, R.D. (2000) Probing proteomes – seeing the whole picture? *Nat. Biotechnol.* 18, 1041–1042
- 15 Justice, M.J. (2000) Capitalizing on large-scale mouse mutagenesis screens. *Nat. Genet.* 1, 109–115
- 16 Goodnow, C.C. *et al.* (1999) Mechanisms of self-tolerance and autoimmunity: from whole animal phenotypes to molecular pathways. *Cold Spring Harbor Symp. Quant. Biol.* 64, 313–322

Yin Liu

Stephen Shaw*

Experimental Immunology Branch, National Cancer Institute, Bethesda, MD 20892, USA.

*e-mail: sshaw@nih.gov

Human mast cells and basophils in HIV-1 infection

Gianni Marone, Giovanni Florio, Angelica Petraroli, Massimo Triggiani and Amato de Paulis

Mast cells and basophils (FcεRI⁺ cells) are classically involved in allergic disorders. HIV-1 glycoprotein gp120 acts as a viral superantigen by interacting with the heavy chain, variable 3 (V_H3) region of IgE to induce cytokine release from FcεRI⁺ cells. The chemokine receptors CCR3 and CXCR4, co-receptors for HIV-1, are expressed by FcεRI⁺ cells. Via its interaction with CCR3, HIV-1 transactivation (Tat) protein is a potent chemoattractant for FcεRI⁺ cells. Incubation of basophils with Tat protein upregulates the surface expression of the CCR3 receptor. There is some evidence that human FcεRI⁺ cells could be infected *in vitro* by M-tropic HIV-1 strains.

Human mast cells and basophils are the only cells that express high-affinity receptors for IgE (FcεRI) and that also synthesize various proinflammatory mediators and cytokines¹. FcεRI⁺ cells play a fundamental role not only in the pathophysiology of allergic disorders, but also in the host's immune response to parasites and bacteria². By contrast, the involvement of FcεRI⁺ cells in viral infections is as yet largely unknown.

The past two decades have seen two apparently unrelated events: AIDS, which is caused by the viruses HIV-1 and HIV-2 (Ref. 3) and a pandemic of allergic disorders, which affects ≈15% of the population of industrialized countries⁴. There is no apparent association between these two disorders: AIDS is an acquired immunodeficiency of viral etiology³ whereas atopic disorders result from the overproduction of IgE in response to allergens⁴. However, serum IgE levels are

increased in HIV-1-infected children⁵ and adults⁶ and the T helper1 (Th1) and Th2 cytokine profile is disturbed in HIV-1 infection because of a shift towards Th2-type cytokines⁷. Moreover, HIV-1-infected patients have an increased prevalence and severity of allergic reactions and adverse reactions to drugs⁸, and HIV-1 antigens induce histamine release from basophils⁹. Here, we review the data that implicate mast cells, basophils and their mediators in HIV-1 infection.

Increased IgE levels in patients with HIV-1 infection

At least two cytokines [interleukin (IL)-4 and IL-13] play a key role in IgE production in humans⁴. IL-4 and IL-13 induce ε germline mRNA expression in B cells, which accounts for the ability of these cytokines to drive the switch to an IgE isotype. IL-4 and IL-13 are produced by Th2 cells⁴, basophils⁹ and mast cells¹⁰. An additional signal for IgE synthesis is provided by the interaction of CD40 expressed by B cells with its natural ligand (CD40L) expressed by activated T cells. Activated mast cells and basophils also express CD40L (Refs 10,11). This finding is consistent with the observation that activated mast cells and basophils, which release IL-4 and IL-13, can induce B cells to synthesize IgE.

IgE levels are elevated in HIV-1-infected children⁵ and adults⁶ and have been associated with disease progression¹². The immune disturbance responsible for the increased IgE levels in HIV-1 infection is not fully understood. Although HIV-1-infected individuals

display polyclonal gammopathy and activated B cells¹³, there is no correlation between IgE levels and either IgG or IgA levels¹².

Th1–Th2 imbalance in HIV-1 infection

Clerici *et al.* provided the first suggestion that, during the early stages of HIV-1 infection, there is a shift from a Th1-type towards a Th2-type pattern of cytokine production by peripheral blood lymphocytes⁷. Increased Th2 cytokine production during HIV-1 infection has been confirmed¹⁴, although not all investigators have detected an overall shift in the cytokine pattern towards the Th2 subset in the lymph nodes or T-cell clones of HIV-1-infected individuals^{15,16}. However, Maggi *et al.* found that HIV-1 replicates preferentially in Th2 as opposed to Th1 clones¹⁵.

These apparently conflicting results concerning the balance between Th1 and Th2 polarization in HIV-1-infected subjects could be caused by diverse factors: technical reasons, the production of Th2-type cytokines by cell types other than lymphocytes (e.g. FcεRI⁺ cells), stimulation by specific HIV-1 superantigens or the effects of cytokines other than IL-4 (e.g. IL-13) on Th2-cell polarization.

HIV-1 gp120 induces IL-4 and IL-13 release from FcεRI⁺ cells

The HIV-1 envelope glycoprotein gp120 mediates entry into immune cells by binding to CD4, the 'primary receptor'¹⁷. CD4 binding causes conformational changes in gp120, resulting in the

exposure and/or formation of binding sites for chemokine receptors (usually CCR5 or CXCR4)¹⁸. Certain strains of viruses can use CCR3, which is also present on human basophils and mast cells¹⁹, as a co-receptor for HIV-1 infection²⁰. These receptor interactions trigger further

conformational changes in gp120 and exposure of the fusion domain of the gp41 subunit, which then mediates fusion of the opposed cell and virus membranes¹⁷.

HIV-1 enters the body predominantly through mucosal surfaces and mast cells are particularly abundant in human mucosa². In addition, the early phases of infection are associated with a high level of viraemia³. Thus, mast cells and basophils can be exposed to shed or virus-bound gp120 during the early phases of HIV-1 infection. IgE-mediated activation of human basophils and mast cells rapidly generates IL-4 and IL-13 (Refs 9,10), which are crucial for Th2-cell polarization⁴.

HIV-1 gp120 is a member of the Ig superantigen family and Ig heavy chain, variable 3 (V_H3) gene products are the ligands for gp120 (Ref. 21). This interaction might explain the superantigenic activation of B cells in patients with AIDS (Ref. 13). We recently demonstrated that four recombinant gp120 variants (gp120_{MN}, gp120_{SF2}, gp120_{LAV} and gp120_{CM}), which were derived from divergent HIV-1 isolates from different viral clades (B and E) of various geographical origins, increased IL-4 and IL-13 mRNA expression and stimulated the release of both cytokines from the basophils of subjects negative for anti-HIV-1 and anti-HIV-2 antibodies⁹. By contrast, interferon (IFN)- γ mRNA was not detected in any of the gp120-stimulated basophil preparations. This provided evidence that gp120-mediated stimulation of Fc ϵ RI⁺ cells induced only cytokines of the Th2 profile. Moreover, these results demonstrate that the capacity to induce Th2-type cytokine release from basophils is a feature of gp120 that is maintained throughout the evolution of HIV.

Removal of the IgE expressed on Fc ϵ RI⁺ cells completely blocked the effect exerted by gp120 on IL-4 and IL-13 secretion from basophils, suggesting that gp120 activates these cells via its interaction with IgE (Ref. 22). Preincubation of gp120 with human monoclonal IgM V_H3 inhibited the effect of gp120 on cytokine secretion from

basophils, whereas monoclonal IgM V_H6 had no effect. Thus, binding to the V_H3 domain inhibits the interaction of gp120 with IgE bound to Fc ϵ RI⁺ cells.

These results are consistent with the hypothesis that HIV-1 gp120 selectively induces the expression of Th2-type cytokines from Fc ϵ RI⁺ cells via the interaction of the superantigen-binding site on gp120 with the V_H3 region of IgE. This interaction could represent an initial and crucial source of IL-4 and IL-13, thereby favouring a shift towards a Th2 phenotype. In advanced HIV-1 infection, when CD4⁺ T-cell numbers are decreased, Fc ϵ RI⁺ cells might also provide a relevant pool of Th2-type cytokines. Therefore, during both early and advanced HIV-1 infection, Fc ϵ RI⁺ cells might be a source of cytokines that contribute to the polarization of CD4⁺ cells towards Th2 cells and increased IgE levels.

Th2 cells represent 0.2–2.0% of CD4⁺ cells, whereas basophils represent 1.0% of peripheral blood leukocytes. Viral antigens interact with individual Th clones. Because the V_H3 family is the largest in the human Ig repertoire ($\approx$ 50%)²¹, shed or virus-bound gp120 could interact with a high frequency with V_H3⁺ IgE bound to Fc ϵ RI⁺ cells. Consequently, the viral superantigen gp120 can produce a rapid and massive activation of Fc ϵ RI⁺ cells via V_H3⁺ IgE.

Cytokines released from Fc ϵ RI⁺ cells might indirectly affect HIV-1 entry into CD4⁺ cells. For example, the upregulation of CXCR4 by IL-4 facilitates HIV-1 infection of T cells²³. This points to an amplification loop of polarized Th2 responses based on the induction of chemokine receptors by gp120-stimulated IL-4 and IL-13 released from Fc ϵ RI⁺ cells.

The evidence that Fc ϵ RI⁺ cells might contribute to the dysregulation of the immune system in HIV-1 infection, by being a relevant source of Th2-type cytokines (IL-4 and IL-13), can explain the observation of viral interaction with CCR3 on human basophils and mast cells^{7,14–16}. It appears that the interaction of a specific viral superantigen, gp120, with cell types other than lymphocytes is important in the production of Th2-type cytokines during HIV-1 infection.

HIV-1 Tat protein activates human Fc ϵ RI⁺ cells

Tat protein is another viral product central to the immunodeficiency in

HIV-1-infected patients. HIV-1 Tat protein can transactivate heterologous viral and cellular genes. It is secreted by HIV-1-infected cells and can activate uninfected cells in a paracrine fashion²⁴.

Tat protein causes chemotaxis of basophils and mast cells from donors negative for antibodies to HIV-1 and HIV-2 via interaction with the CCR3 receptor²⁵. Preincubation of Fc ϵ RI⁺ cells with Tat protein upregulates the surface expression of CCR3 and increases the expression of specific CCR3 mRNA. CCR3 is a co-receptor for several strains of HIV-1 (Ref. 20), and thus upregulation of CCR3 on basophils and mast cells by Tat could facilitate HIV-1 infection of Fc ϵ RI⁺ cells. It is noteworthy that the chemokine receptor CXCR4, a primary co-receptor for HIV-1, is functionally expressed on human Fc ϵ RI⁺ cells²⁶. Consequently, during HIV-1 infection, which is associated with high Tat levels in serum and other biological fluids²⁴, Tat can influence the directional migration of Fc ϵ RI⁺ cells, thereby contributing to the recruitment of these cells to sites of HIV-1 infection. Moreover, preincubation of basophils from healthy individuals with IL-3 potentiates Tat-induced IL-4 and IL-13 synthesis. Thus, Tat is the first virokin (β-chemokine homolog) encoded by a retrovirus that has been shown to contribute to the release of Th2-type cytokines from Fc ϵ RI⁺ cells during HIV-1 infection.

Thus, at least two viral proteins, gp120 and Tat, can induce the production of IL-4 and IL-13 by human Fc ϵ RI⁺ cells, which suggests that Fc ϵ RI⁺ cells might be a novel source of Th2 cytokines, thereby contributing directly and indirectly to the immune dysregulation in HIV-1 infection. Box 1 summarizes the findings that support the role of Fc ϵ RI⁺ cells in HIV-1 infection.

Can certain strains of HIV-1 infect human mast cells and basophils?

The finding that the HIV-1 proteins gp120 and Tat can interact with different surface molecules on Fc ϵ RI⁺ cells and that they can upregulate two chemokine receptors (CCR3 and CXCR4)^{23,25} that act as co-receptors for HIV-1 (Refs 18,20), prompted the provocative suggestion that mast cells and basophils might be a novel reservoir of HIV-1 infection.

Li *et al.* showed that a population of mast cells and/or basophils in the

peripheral blood of allergic patients expressed CD4, CCR3, CCR5 and CXCR4 (Ref. 27). They also reported that this population of cells can be infected *in vitro* by M-tropic strains of HIV-1. It is therefore conceivable that mast cell and/or basophil progenitors present in peripheral blood could be infected by M-tropic HIV-1 strains. However, it remains to be established whether or not this finding applies to the *in vivo* condition.

Conclusions and perspective

In asking whether human FcεRI⁺ cells could be directly and/or indirectly involved in HIV-1 infection, we found that gp120 from different clades stimulates IL-4 and IL-13 release from basophils and mast cells via gp120 interaction with the V_H3 region of IgE. Because the V_H3 family is the largest of the human Ig repertoire, shed or virus-bound gp120 probably interacts with a high frequency with V_H3⁺ IgE bound to FcεRI⁺ cells of normal individuals or individuals at an early stage of infection. HIV-1 Tat protein also induces IL-4 and IL-13 release from basophils. Thus, the HIV-1 proteins gp120 and Tat can induce the synthesis of Th2 cytokines, thereby favouring a shift towards a Th2 phenotype. IL-4 and IL-13 induced by gp120 and by Tat might also interact with specific receptors on B cells, in turn inducing ε germline mRNA expression and so causing a switch to the IgE isotype. This sequence of events, schematically represented in Fig. 1, could well explain the elevated IgE levels in HIV-1-infected children and adults^{5,6}.

Human FcεRI⁺ cells could also be involved in HIV-1 infection in other ways. In fact, Tat protein, secreted by HIV-1-infected cells²⁴, is a potent chemoattractant for FcεRI⁺ cells and upregulates the expression of CCR3 on their surface²⁵. Human mast cells and basophils express CCR3 (Ref. 19), a receptor that can be used by several strains of HIV-1 (Ref. 20). Cord-blood-derived mast cells, mature mast cells and basophils express CXCR4 (Refs 22,27), a co-receptor for HIV-1 (Ref. 18). Finally, the finding that mast cell and/or basophil precursors can be infected *in vitro* by M-tropic HIV-1 strains impinges on the accepted scheme of HIV-1 pathophysiology.

Box 1. A summary of the evidence that human mast cells and basophils are involved in HIV-1 infection

- HIV-1 gp120 induces histamine and cysteinyl leukotriene C 4 (LTC₄) release from human basophils and mast cells of normal donors negative for antibodies to HIV-1 and HIV-2.
- HIV-1 gp120 induces Th2-type cytokine (IL-4 and IL-13) release from human basophils of normal donors negative for antibodies to HIV-1 and HIV-2 via interaction with the V_H3 region of IgE.
- HIV-1 Tat protein is chemotactic for human basophils and mast cells.
- HIV-1 Tat protein upregulates CCR3 receptors on human basophils and mast cells.
- HIV-1 Tat protein induces IL-4 and IL-13 release from human basophils of normal donors negative for antibodies to HIV-1 and HIV-2.
- Cord-blood-derived mast cells express CXCR4, CCR5 and CCR3 receptors.
- CCR3 and CXCR4 receptors are constitutively expressed by human mast cells and basophils.
- Human mast cell and/or basophil precursors can be infected *in vitro* by M-tropic HIV-1 strains.

One can envisage that infected FcεRI⁺ cells carry HIV-1 to lymph node lymphocytes and across the blood–brain barrier to microglia and astrocytes. Interestingly, mast cell density is increased in lymph nodes from AIDS patients²⁸ and mast cells are one of the few types of immune cell in the brain²⁹.

In conclusion, the HIV-1 proteins gp120 and Tat can activate human

FcεRI⁺ cells via several different immunological mechanisms, so inducing the release of Th2 cytokines. Tat protein upregulates the CCR3 receptor on FcεRI⁺ cells. Human mast cell and/or basophil precursors also express the CXCR4 and CCR5 receptors and can be infected *in vitro* by M-tropic HIV-1 strains. These observations suggest a novel and complex role for FcεRI⁺ cells in HIV-1 infection.

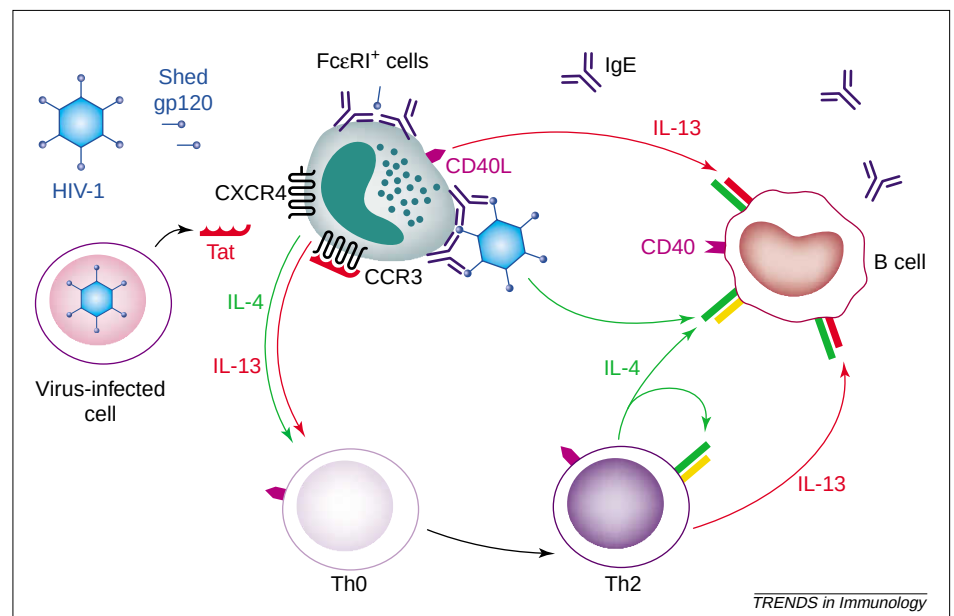


Fig. 1. Schematic representation of some of the interactions between HIV-1 and human mast cells and basophils (FcεRI⁺ cells). Shed- or virus-bound-gp120 can interact with variable, heavy chain 3 (V_H3)⁺ IgE bound to FcεRI⁺ cells of individuals at an early stage of infection. This induces interleukin (IL)-4 and IL-13 release and favours a shift towards a T helper 2 (Th2) phenotype. IL-4 and IL-13 might also interact with specific surface receptors so inducing ε germline mRNA expression in B cells and a switch to the IgE isotype. HIV-1 transactivation (Tat) protein secreted by infected cells interacts with the surface chemokine receptor CCR3 and is a potent chemoattractant for human basophils and mast cells. Extracellular Tat can also induce IL-4 and IL-13 release from these cells. Activated mast cells and basophils express CD40 ligand (CD40L). CD40L–CD40 interaction can induce B cells to synthesize IgE. Human FcεRI⁺ cells express CCR3 and CXCR4, which are co-receptors for several strains of HIV-1, suggesting that these cells could be infected by certain strains of HIV-1.

Acknowledgements

We dedicate this paper to Rita Levi-Montalcini who first suggested that FcεRI⁺ cells were involved in HIV-1 infection. The Istituto Superiore Sanità (AIDS project 40B.64 and 40A.67), CNR (Target project Biotechnology No. 99.00216.PF31 and No. 99.00401. PF49) and MURST are acknowledged for supporting our work.

References

- Marone, G. *et al.*, eds (2000) *Mast Cells and Basophils*, Academic Press
- Patella, V. *et al.* (2000) Human mast cells and basophils in immune response to infectious agents. In *Mast Cells and Basophils* (Marone, G. *et al.*, eds), pp. 397–418, Academic Press
- Fauci, A.S. (1999) The AIDS epidemic. Considerations for the 21st century. *New Engl. J. Med.* 341, 1046–1050
- Romagnani, S. (2000) The role of lymphocytes in allergic disease. *J. Allergy Clin. Immunol.* 105, 399–408
- Viganò, A. *et al.* (1995) Elevation of IgE in HIV-infected children and its correlation with the progression of disease. *J. Allergy Clin. Immunol.* 95, 627–632
- Lucey, D.R. *et al.* (1990) Serum IgE levels in 622 persons with human immunodeficiency virus infection: IgE elevation with marked depletion of CD4⁺ T cells. *AIDS Res. Hum. Retroviruses* 6, 427–429
- Clerici, M. and Shearer, G.M. (1993) A Th1–Th2 switch is a critical step in the etiology of HIV infection. *Immunol. Today* 14, 107–111
- Kovacs, J.A. *et al.* (1984) *Pneumocystis carinii* pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. *Ann. Intern. Med.* 100, 663–671
- Patella, V. *et al.* (2000) HIV-1 gp120 induces IL-4 and IL-13 release from human FcεRI⁺ cells through interaction with the V_H3 region of IgE. *J. Immunol.* 164, 589–595
- Gauchat, J.-F. *et al.* (1993) Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 365, 340–343
- Pawankar, R. *et al.* (1997) Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the FcεRI, CD40L, IL-4 and IL-13, and can induce IgE synthesis in B cells. *J. Clin. Invest.* 99, 1492–1499
- Israël-Biet, D. *et al.* (1992) Elevation of IgE in HIV-infected subjects: a marker of poor prognosis. *J. Allergy Clin. Immunol.* 89, 68–75
- Schnittman, S.M. *et al.* (1986) Direct polyclonal activation of human B lymphocytes by the acquired immune deficiency syndrome virus. *Science* 233, 1084–1086
- Meyaard, L. *et al.* (1994) Changes in cytokine secretion patterns of CD4⁺ T-cell clones in human immunodeficiency virus infection. *Blood* 84, 4262–4268
- Maggi, E. *et al.* (1994) Ability of HIV to promote a Th1 to Th0 shift and to replicate preferentially in Th2 and Th0 cells. *Science* 265, 244–248
- Graziosi, C. *et al.* (1994) Lack of evidence for the dichotomy of Th1 and Th2 predominance in HIV-infected individuals. *Science* 265, 248–252
- Kwong, P.D. *et al.* (1998) Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* 393, 648–659
- Moore, J.P. *et al.* (1997) Coreceptors: implications for HIV pathogenesis and therapy. *Science* 276, 51–52
- Romagnani, P. *et al.* (1999) Tryptase-chymase double-positive human mast cells express the eotaxin receptor CCR3 and are attracted by CCR3-binding chemokines. *Am. J. Pathol.* 155, 1195–1204
- Choe, H. *et al.* (1996) The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 85, 1135–1148
- Karray, S. and Zouali, M. (1997) Identification of the B cell superantigen-binding site of HIV-1 gp120. *Proc. Natl. Acad. Sci. U. S. A.* 94, 1356–1360
- Florio, G. *et al.* (2000) The immunoglobulin superantigen-binding site of HIV-1 gp120 activates human basophils. *AIDS* 14, 931–938
- Galli, G. *et al.* (1998) Enhanced HIV expression during Th2-oriented responses explained by the opposite regulatory effects of IL-4 and IFN-γ on fusin/CXCR4. *Eur. J. Immunol.* 28, 3280–3290
- Ensoli, B. *et al.* (1990) Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. *Nature* 345, 84–86
- de Paulis, A. *et al.* (2000) Tat protein is an HIV-1 encoded β-chemokine homolog that promotes migration and upregulates CCR3 expression on human FcεRI⁺ cells. *J. Immunol.* 165, 7171–7179
- Jinquan, T. *et al.* (2000) Chemokine stromal cell-derived factor 1α activates basophils by means of CXCR4. *J. Allergy Clin. Immunol.* 106, 313–320
- Li, Y. *et al.* (2000) Identification of a novel population of mast cells/basophils in human peripheral blood of allergic patients which is HIV-1 susceptible due to its surface expression of CD4, CCR5 and CXCR4. *J. Allergy Clin. Immunol.* 105, S86
- Paiva, D.D. *et al.* (1996) Spectrum of morphologic changes of lymph nodes in HIV infection. *Mem. Inst. Oswaldo Cruz* 91, 371–379
- Silver, R. *et al.* (1996) Mast cells in the brain: evidence and functional significance. *Trends Neurosci.* 19, 25–31

Gianni Marone*

Giovanni Florio

Angela Petraroli

Massimo Triggiani

Amato de Paulis

Division of Clinical Immunology and Allergy,
University of Naples Federico II,
Via S. Pansini 5, 80131 Napoli, Italy.

*e-mail: marone@unina.it

NO-releasing NSAIDs are caspase inhibitors

Stefano Fiorucci

Nitrosation of thiol-containing proteins is a mechanism for cell regulation. Nitric-oxide-releasing nonsteroidal anti-inflammatory drugs (NO-NSAIDs) are chemical entities obtained by adding a nitroxybutyl moiety to a conventional NSAID. NO-NSAIDs inhibit inflammation via cyclo-oxygenase (COX)-dependent and -independent effects. Similarly to endogenous NO, NO-NSAIDs are potent inhibitors of T helper1 (Th1) type cytokines. This effect is largely owing to post-translational nitrosation and therefore inactivation of cysteine proteases, such as the interleukin (IL)-1β converting enzyme (ICE/caspase-1) involved in pro-cytokine processing.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are effective for the treatment of pain, inflammation and fever, and aspirin is increasingly used for prophylaxis of vascular disorders. However, the use of NSAIDs is associated with significant gastro-intestinal (GI) toxicity. Today, NSAIDs are the most common cause of gastro-duodenal injury in western countries. The main mechanism of action of NSAIDs is inhibition of prostaglandin-forming cyclo-oxygenases (COXs). Two COX isoforms are involved in prostaglandin synthesis: COX-1 is constitutively expressed and generates prostaglandins

involved in GI mucosal protection and platelet function, whereas COX-2 is induced at sites of inflammation to generate prostaglandins that mediate inflammation. The anti-inflammatory effects of nonselective NSAIDs are mediated via COX-2 inhibition, whereas the harmful effects are believed to be caused primarily by COX-1 inhibition. Several strategies have been developed to decrease the risk of NSAID-associated GI disorders. The development of safer NSAIDs, COX-2-selective inhibitors and nitric-oxide-releasing NSAIDs (NO-NSAIDs) has been demonstrated to be both rational and successful. COX-2