Are Basophils and Mast Cells Masters in HIV Infection?

Gianni Marone\textsuperscript{a–c}, Gilda Varricchi\textsuperscript{a, b}, Stefania Loffredo\textsuperscript{a, b}, Maria Rosaria Galdiero\textsuperscript{a, b}, Felice Rivellese\textsuperscript{d} and Amato de Paulis\textsuperscript{a, b}

\textsuperscript{a} Department of Translational Medical Sciences (DiSMeT) and \textsuperscript{b} Center for Basic and Clinical Immunology Research (CISI), University of Naples Federico II, and \textsuperscript{c} Institute of Endocrinology and Experimental Oncology (IEOS), CNR, Naples, Italy; \textsuperscript{d} William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

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Abstract
The World Health Organization AIDS epidemic update estimates that more than 37 million people are living with HIV infection. Despite the unprecedented success of antiretroviral treatments, significant challenges remain in the fight against HIV. In particular, how uninfected cells capture HIV and transmit virions to target cells remains an unanswered question. Tissue mast cells and peripheral blood basophils can be exposed to virions or HIV products during infection. Several HIV proteins (i.e., envelope glycoproteins gp120 and gp41, Tat, and Nef) can interact with distinct surface receptors expressed by human basophils and mast cells and modulate their functional responses at different levels. Additionally, several groups have provided evidence that human mast cells can be infected in vitro, as well as in vivo, by certain strains of HIV. Recently, it has been demonstrated that basophils purified from healthy donors and intestinal mast cells can efficiently capture HIV on their cell surface and, cocultured with CD4\textsuperscript{+} T cells, they can transfer the virus to the cocultured cells leading to infection. Direct contact between human basophils or intestinal mast cells and CD4\textsuperscript{+} T cells can mediate viral trans-infection of T cells through the formation of viral synapses. Thus, basophils and mast cells can provide a cellular basis for capturing and then spreading viruses throughout the body. Collectively, these findings suggest that human basophils and mast cells play a complex and possibly distinct role in HIV infection, warranting further investigations.

Introduction
The UNAIDS/World Health Organization AIDS epidemic update estimates that more than 37 million people were living with HIV in 2015 (global AIDS update 2016, http://www.unaids.org). New infections have been occurring in the past few years at a rate of 2.1 million/year, and almost 1.1 million individuals have succumbed to AIDS-related diseases. Although many people are now receiving life-saving antiretroviral treatment (an estimate of 17 million in 2015, and these have been very effective in reducing AIDS-related deaths, i.e., from 1.5 million in 2010 to 1.1 million in 2015), significant challenges remain in the fight against HIV infection and many aspects of its
Pathogenesis still need to be clarified [1, 2]. Most infections are nowadays acquired through sexual intercourse by means of mucosal transmission [3]. During acute HIV infection, patients experience high levels of plasma viremia, causing vigorous, though inadequate, cellular and humoral immune responses. Neutralizing antibodies against cell-free virus are detected in the sera of infected individuals, but these are generally ineffective against circulating HIV [4, 5]. How uninfected cells capture HIV and transmit the virus to target cells via the virological synapse or how HIV infects target cells and replicates, persisting in the face of a vigorous immune response, remains unanswered [6, 7].

HIV entry into CD4+ T cells is mediated by the virus-encoded envelope glycoproteins gp120 and gp41. These proteins interact with the primary cellular receptor CD4 and its coreceptors (e.g., CCR5, CXCR4, or CCR3), which triggers the fusion of the viral and host cell membranes [8]. However, the details of viral propagation and spread between cells and host tissues are still poorly characterized. Expanding our knowledge on these aspects is essential to understanding the pathogenesis of HIV infection and its progression to AIDS.

Human basophils and mast cells are characterized by expression of the high affinity receptor for IgE (FceRI) and by the unique ability to release preformed and de novo synthesized mediators [9–11]. Despite some similarities, there are striking differences in the morphological, biochemical, and immunological characteristics of human basophils and mast cells (Table 1) and in their roles played in innate and adaptive immunity [12, 13]. Although the primary location of human basophils is the peripheral blood, under certain pathological conditions basophils are recruited into inflamed tissues [14–16] and in lymph nodes [17, 18].

Human mast cells and basophils have long been regarded as critical effector cells in IgE-associated allergic disorders [10, 11]. However, there is now evidence that mast cells and basophils are versatile effector and regulatory cells able to initiate and modulate both innate and adaptive immune responses to parasites and bacterial pathogens [12, 19, 20]. Similarly, basophils and mast cells have been involved in many physiological and pathological processes, such as autoimmunity [21, 22] and cancer [10], where they can play multifaceted roles with both pro- and anti-inflammatory/immunomodulatory func-

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The role of basophils and mast cells in viral infections is more enigmatic and has been less well studied [13]. In particular, the extent of mast cell/basophil involvement in the response to HIV infection and the resulting pathophysiological implications have not been thoroughly investigated [24–26].

The majority of HIV infections are nowadays acquired by mucosal exposure, with sexual transmission as the leading mode of infection worldwide [3]. Mast cells can be found in almost all vascularized tissues, but they are most abundant beneath epithelial and mucosal surfaces. In addition, early phases of HIV infection are associated with a high level of viremia [1, 27]. Thus, mast cells and basophils can be exposed to virions or viral products during the early phases of HIV infection, the former at the site of viral entry and the latter in circulation during the viremic phase.

**Effects of HIV Proteins on Human Basophils and Mast Cells**

In the course of HIV infection, the gp160 envelope glycoprotein is enzymatically cleaved, yielding 2 proteins, i.e., the transmembrane gp41 and the surface gp120 [28]. In the early events of HIV infection, gp120 binds to CD4 on immune cells, causing conformational changes in the envelope glycoprotein. This event induces exposure of binding sites for chemokine receptors (CCR5 or CXCR4) that serve as obligated coreceptors for virus entry [29, 30]. Certain strains of virus can use CCR3 present on human basophils [31] and mast cells [31, 32] as the coreceptor for HIV infection [33, 34]. The interaction with coreceptors induces further conformational changes in the envelope glycoprotein and exposure of the fusion domain of the gp41 subunit that mediates the fusion of the target cell and virus membranes [35, 36].

gp120 is a member of the immunoglobulin superantigen family [37] and binds to the V_3 region of human immunoglobulins. These findings could explain the superantigenic activation of B lymphocytes observed in patients with HIV infection [38]. gp120 isolated from different HIV strains of various geographical origins was shown to induce IL-4 and IL-13 production from basophils purified from healthy donors negative for HIV-1 and HIV-2 antibodies [39, 40]. gp120 activates basophils through the interaction with IgE V_3 since removal of IgE completely blocks the envelope glycoprotein’s effect on IL-4 and IL-13 release. During the early phase of HIV infection associated with a high level of viremia [1, 41], basophils can be exposed to virus-bound or shed gp120. This may be an initial and critical source of IL-4 and IL-
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13, thereby favoring a shift toward a Th2 phenotype [42], and it might explain the elevated IgE concentration in HIV-infected adults and children [43, 44]. IL-4 release from basophils might also indirectly affect HIV entry into CD4+ cells. Indeed, IL-4 upregulates CXCR4 [8] and facilitates HIV infection of T cells [45].

gp120 proteins from different HIV subtypes bind to the heterodimeric α4β7 integrin [46]. This interaction contributes to generating the virological synapses in HIV infection between dendritic cells and CD4+ cells or between donor and targeted CD4+ cells [7]. Virological synapses facilitate the efficient cell-to-cell transfer of virions in HIV infection.

gp41 is thought to mediate the fusion between viral and cellular membranes by insertion of a hydrophobic N-terminus into the plasma membrane [28]. gp41 also exerts multiple effects on the host immune system. We found that envelope gp41, as well as several synthetic peptides encompassing its structure, promote the migration of human basophils through the interaction with formyl peptide receptors [47]. Together, these findings indicate the complexity of the interactions between HIV envelope peptides (i.e., gp120 and gp41) and different receptors expressed by human basophils.

The HIV trans-activator protein (Tat), secreted by HIV-infected cells [48–50], can be taken up by neighboring cells [51] in which it can reactivate a latent infection [50]. Thus, extracellular Tat mimics some of the effects of HIV on immune cells, suggesting an important role for this protein in the pathogenesis of HIV infection. We found that Tat is a potent chemoattractant for human basophils and mast cells obtained from healthy individuals through engagement of the CCR3 receptor expressed on these cells [52]. Thus, during HIV infection, associated with a high level of Tat in serum and other biological fluids [48], Tat induces the directional migration of FcεRI+ cells, thereby contributing to their recruitment to sites of HIV infection.

The nef gene is only present in the genomes of primate lentiviruses (i.e., HIV-1, HIV-2, and SIV). The protein is translated from a multiply spliced mRNA and it is highly expressed from the early stages of HIV infection. HIV Nef is required to maintain a high virus production within infected cells [53], and the loss of Nef results in delayed or absent disease progression [54]. Nef does not possess enzymatic activity, but it exerts its cellular functions by modulating the expression of several cell surface molecules [55]. Recently, de Paulis and collaborators found that Nef proteins, in concentrations similar to the ones found in the sera of infected patients [56], influences the directional migration of basophils isolated from healthy subjects via interaction with the chemokine receptor CXCR4 [57].

Thus, at least 4 HIV proteins, i.e., gp120, gp41, Tat, and Nef, were shown to interact with specific surface receptors expressed by human basophils and mast cells and can modulate several functional activities of these cells (Fig. 1).

Can Human Basophils and Mast Cells Be Infected by HIV?

The finding that several HIV proteins can interact with different surface receptors on human basophils and mast cells [24, 39, 40, 47, 52, 58] prompted the intriguing hypothesis that these cells may be a reservoir of HIV infection. The group of Krilis reported that a population of mast cells/basophils in the peripheral blood of allergic patients can be infected in vitro by M-tropic strains of HIV [59]. Another group showed that human mast cell progenitors (CXCR4*, CCR5*, and CCR3*) can be infected in vitro by M-tropic, but not T-tropic, viruses [60]. Similarly, HIV-infected mast cells/basophils were found in the peripheral blood of AIDS patients [61]. Sundstrom et al. [62] reported that progenitor mast cells developed in vitro from CD34+ cord blood stem cells can be experimentally infected with CCR5-tropic strains of HIV. In addition, they found that infected mast cells, particularly when triggered though Toll-like receptors, were capable of establishing productive infection in target cells. These results suggest that human mast cells may serve both as a viral reservoir and as a model of latency in HIV infection. Certain strains of HIV can infect also the human mast cell line HMC-1 through the interaction with CXCR4 [63]. In addition, mast cells cultured in vitro from circulating CD34+ pluripotent progenitors, but not mature mast cells, are susceptible to HIV infection [64]. Importantly, the authors provided evidence that placental mast cells isolated from HIV-infected women harbor HIV. The same group reported that progenitor mast cells comprise a reservoir of HIV infection. In conclusion, progenitor mast cells derived from CD34+ precursors are susceptible in vitro to CCR5(R5)-tropic viruses, but only marginally to CXCR4(X4)-tropic HIV [65]. In conclusion, it is conceivable that human mast cell and/or basophil progenitors present in peripheral blood could be infected by certain strains of HIV, although it remains to be confirmed whether these findings are also true in vivo, as for example the active virus replication in tissue mast cells has not been confirmed [66].
Can Human Basophils and Mast Cells Capture HIV and Mediate Viral Infection of Other Immune Cells?

In order to gain a foothold in an uninfected host, cell-free or cell-associated HIV must infect target cells at, or proximal to, the portal of entry into the body and subsequently disseminate throughout the body [67]. HIV infection of target cells can occur via 2 different mechanisms: HIV can infect target cells (i.e., dendritic cells) and replicate, producing virions which in turn infect new target cells (i.e., CD4 + T cells) (cis-transmission). An alternative and probably more efficient mechanism is trans-transmission through the virological synapse [68, 69]. In this process, uninfected cells capture HIV and transmit virus to target cells via the virological synapse or via HIV-bearing exosomes [6]. In the latter mechanism, there is no viral replication in the original cells and the process is associated with polarization of specific receptors (i.e., α4β7, CXCR4, CCR5, and CD4) on target cells [6, 7]. Cell-associated HIV infection (i.e., trans-transmission) has been shown to play a pivotal role in HIV spread [70–72].

Jiang et al. [73] elegantly demonstrated that basophils purified from healthy donors capture HIV and mediate viral trans-infection of CD4 + T cells. First, they confirmed that human basophils express CXCR4 and CCR5. Then, they found that human basophils efficiently capture HIV particles on the cell surface. Interestingly, basophils, but not neutrophils, perform viral capture efficiently. They also confirmed [39, 40] that gp120 binds to basophils. Importantly, they found that purified basophils pulsed with HIV and then cocultured with CD4 + T cells were able to transfer HIV to the cocultured cells, leading to robust infection. No viral replication was observed in basophils, suggesting that surface-bound HIV particles play the greatest role in viral transfer. Using confocal microscopy, they found that contact between basophils and CD4 + T cells is required for viral transfer. More recently, the same group extended the previous observations showing that also human intestinal mast cells can capture HIV and mediate viral trans-infection of CD4 + T cells [74]. Collectively, these fascinating results suggest that direct contact between basophils/mast cells and CD4 + T cells and the formation of infection synapses facilitate trans-infection of HIV particles. In conclusion, these studies suggest that circulating naïve basophils from early-infected donors can capture HIV particles and mediate viral trans-infection of encountered CD4 + T cells. Thus, basophils can provide a cellular basis for capturing and then spreading viruses throughout the body. The mechanism by which HIV is captured by human basophils/mast cells may involve the superantigenic interaction between IgE V_{H}3 + and virus-bound gp120 [39, 40]. In addition, gp120 glycoproteins from different HIV subtypes bind to integrin α4β7 [46], which is highly expressed by human basophils [73] and CD4 + T cells [46] (Fig. 1).

Jiang et al. [73] also found that the basophil count remains fairly stable during HIV progression. This observation suggests that basophils might provide a stable cellular source for viral capture and efficient cell-to-cell transfer of virions during HIV infection. Collectively, these interesting results offer novel insights into the role of human basophils/mast cells in HIV dissemination.

Differential Role of Human Basophils and Mast Cells in HIV Infection?

The erroneous view that basophils are a minor and redundant variant of tissue mast cells was held for several decades [12, 13, 15]. Despite some similarities (i.e., the expression of FcεRI and the release of several chemical mediators), human basophils and mast cells display striking differences in their development, life cycle, and fundamental immunological characteristics (Table 1). For example, human basophils produce large quantities of a restricted profile of Th2-like cytokines (IL-4/IL-13 and IL-3) in response to different stimuli [58, 75–78], while human mast cells produce a much wider range of cytokines/chemokines and proinflammatory mediators. In addition, human basophils synthesize only the proangiogenic VEGF-A [79], while mast cells produce both angiogenic and lymphangiogenic molecules [80, 81]. The differences between basophils and mast cells have been recently confirmed by evidence that human mast cells possess a unique expression profile, sharing only few transcripts with basophils [82]. Therefore, it is likely that basophils and mast cells play distinct roles in different physiopathological conditions [10, 12, 13].

Although the physiological role of mast cells and basophils remains enigmatic, there is now increasing evidence that these cells are critically involved not only in allergic disorders but also in autoimmune and infectious diseases, immunodeficiencies, and cancer [13–15, 17, 83], with multifaceted roles due to their ability to mediate both pro- and anti-inflammatory/immunomodulatory responses [21, 23, 84].

Regarding their role in HIV infection, human mast cells can be infected in vitro and probably in vivo by certain HIV strains [59–65]. By contrast, there is no evidence...
that human basophils can be infected by HIV [73]. However, recent evidence suggests that human basophils and mast cells can capture HIV and can mediate viral trans-infection of CD4+ T cells [73, 74]. A strategy designed to prevent basophil/mast-cell-mediated viral capture and transfer may represent a novel direction for the development of anti-HIV treatment.

Taken together, these observations suggest complex, but probably distinct, roles for basophils and mast cells in HIV infection, which deserve further investigations.

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We dedicate this paper to the memory of Rita Levi-Montalcini, who first suggested an involvement of mast cells and basophils in HIV infection.

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