Leukocyte circulation: one-way or round-trip? Lessons from primary immunodeficiency patients

Raffaele Badolato
Department of Pediatrics, University of Brescia, Italy

Abstract: The identification of chemokines has profoundly changed the way we interpret the immune response, elucidating the mechanism by which inflammatory cells are recruited to the site of infection by local secretion of chemoattractants such as CXC chemokine ligand 8 (CXCL8)/interleukin-8, chemokine ligand 2 (CCL2)/monocyte chemoattractant protein 1. This novel view of the immune response has been remodeled further following observations that lymphoid tissue development derives from the coordinated secretion of homeostatic chemokines such as CCL19, CCL21, and CXCL13, which mediate recruitment and clustering of the cells involved in lymphoid organogenesis. The study of primary immunodeficiencies has demonstrated that the number of circulating leukocytes is dependent on migration amongst bone marrow, blood circulation, and inflamed tissues. Defects of leukocyte adhesion and chemotaxis as a result of mutations of β2-integrins lead to abnormal leukocytosis and susceptibility to skin infections, as observed in leukocyte adhesion deficiency. Conversely, neutropenia in children with myelokathexis is a result of leukocyte retention in the bone marrow because of the mutations of CXC chemokine receptor 4, which affect the capacity of cells to recirculate between blood and bone marrow. Moreover, the identification of the genetic basis of primary immunodeficiencies has shown that many primary immunodeficiencies such as Wiskott-Aldrich syndrome and common variable immunodeficiencies are characterized by altered migration of leukocytes and/or disregulation of cellular response to chemokines. This paper will be focused on the interpretation of primary immunodeficiencies as defects in leukocyte circulation between blood and primary and secondary organs. J. Leukoc. Biol. 76: 1–6; 2004.

Key Words: Wiskott-Aldrich syndrome · WHIM · CXCR4 · chemokines · β-integrins

INTRINSIC DEFECTS OF LEUKOCYTE MOTILITY: LEUKOCYTE ADHESION DEFICIENCY (LAD) AND WISKOTT-ALDRICH SYNDROME (WAS)

Before chemokines and chemokine receptors were identified [1–3], Springer et al. [4] had described children with defective adhesion and migration to inflammatory stimuli. This condition, known as LAD, is characterized by recurrent skin infections without pus production despite the chronic leukocytosis. The number of circulating neutrophils in LAD patients is increased, as their cells fail to adhere to the endothelium of blood vessels, a requirement for extravasating to tissues. The process of leukocyte extravasation is dynamic and involves a first step of rolling mediated by various selectins such as CD15A, followed by firm adhesion based on activation of β2-integrins. Chemokines presented on cell surface by vascular endothelium trigger integrin avidity to endothelial ligands to induce successful arrest on target endothelial sites [5]. In the last phase of this process, neutrophils transmigrate along the chemokine gradient to the inflammatory site. Patients affected by type-1 LAD (LAD-1) have a selective defect in the expression and/or functional activation of β2-integrins by neutrophils and monocytes, which result from heterogeneous mutations of the β2-suhunit CD18 [4, 6]. Neutrophilia is associated to increased granulocyte-colony stimulating factor (G-CSF) and interleukin (IL)-17 serum concentrations in CD18−/− mice, suggesting that disruption of leukocyte circulation also leads to increased granulocytopenia [7]. Another rare defect of leukocyte migration is the LAD-2, which is characterized by defective rolling of leukocytes as a result of lack of fucosylated glycoconjugates such as CD15A and other selectins, which are involved in the primary interactions between blood vessel endothelium and circulating leukocytes [8–10]. A functional defect in activation of β1- and β2-integrins avidity to their endothelial ligands by chemokines displayed on the cell surface of vascular endothelium is observed in LAD-3, thereby leading to defective leukocyte arrest on vascular endothelium and leukocytosis, which is observed in this condition [11]. The investigation of these three distinct defects in leukocyte adhesion suggests that the most prominent functions of β2-integrins and selectins are related to migration of leukocytes to the site of inflammation where proinflammatory chemokines such as CXC chemokine ligand 8 (CXCL8) and chemokine ligand 2 (CCL2) are secreted. Nevertheless, the heterodimer CD11c/CD18 is not selectively expressed by phagocytic cells but is also detectable in cells engaged in processing foreign antigens such as myeloid dendritic cells (DCs), suggesting a potential role of CD18 in migration of cells required to initiate the...
antigen, undergo functional maturation, which enables them to present antigen entry following capture and processing of the antigen, undergo functional maturation, which enables them to migrate and locate to the regional lymph nodes to activate antigen-specific T cells. Co-workers and I [13] have recently demonstrated that myeloid DCs derived from LAD-1 patients have a severe impairment in endothelium transmigration and in their chemotactic response, indicating that the immunological defect of CD18-deficient children probably involves T and B cell activation by DCs. This observation may account for the profound alteration in the architectural structure of their lymph nodes and secondarily, for their defective delayed type hypersensitivity (DTH) reactions and the diminished T and B cell functions reported by many authors in LAD-1 patients [14].

This observation raises the question whether impairment of DTH response in other primary deficiencies such as WAS is related to defects in leukocyte motility [15]. In WAS patients, the impaired DTH response is associated with bacterial and viral infections and abnormalities in the cellular and humoral arms of the immune system. During the first decade of life, WAS patients display a progressive decline in the number of circulating T cells and an inability to mount a response against polysaccharidic antigens (see ref. [16] for review). The gene responsible for the disease encodes for an intracellular protein, WAS protein (WASp), which is exclusively expressed in hematopoietic cells. This discovery has focused the attention of researchers on various abnormalities in the cytoskeletal architecture of T cells and monocytes of WAS patients but did not explain the abnormalities in the humoral and cell-mediated arms of the immune system. Several reports have demonstrated that monocytes, macrophages, DCs, and T cells of WAS patients show a reduced migration in response to the chemokines CCL2, CCL3, and CXCL12 [17, 18]. These findings further support the view that WASp interacts with several cytoskeletal components such as the actin-related protein complex (Arp2/3) and with the small guanosine 5'-triphosphate-binding proteins cdc42, both of which are involved in the rearrangement of leukocyte cytoskeleton in response to chemokines. Other authors and I have reported an impaired chemotactic activity of immature DCs and T cells of WAS patients; this defect accounts for the susceptibility to viral and bacterial infection and for the absence of DTH response in WAS patients but does not completely explain the mechanism of the progressive decline in T cell numbers [19, 20]. Recently, Siminovitch and co-workers [21] have shown that mice with a deletion of the WASp, which is required for interaction with the Arp2/3 complex, exhibit an early block of T lymphopoiesis and fail to generate single-positive CD4 and CD8 cells. This suggests that thymocyte differentiation is associated with events that require actin polymerization, such as migration toward and/or interaction with thymic epithelial and stromal cells. Generation of mature T cells in the thymus is inhibited in pertussis toxin transgenic mice, suggesting an involvement of G-protein-coupled receptors in the developmental process [22].

The homeostatic chemokines CCL19, CXCL12, and CCL21 are expressed in the thymic medulla but also at very high levels in the lymph nodes and the spleen. These chemokines regulate the movement of positively selected, mature T cells to the thymic medulla and subsequently, to secondary lymphoid tissues [23–25]. Conversely, the patterns of chemokine responsiveness of thymocytes are dramatically different at various stages of thymic development, suggesting that expression of chemokine receptors and development of functional response to chemokines antecede migration from cortical thymus to medulla [26]. Therefore, defective motility of thymocytes in WAS patients may interfere with the ability of these cells to undergo maturation.

FATAL ATTRACTION OF NEUTROPHILS TO BONE MARROW OF WARTS, HYPOGAMMAGLOBULINEMIA, INFECTIONS, AND MYELOKATHESIS (WHIM) PATIENTS

Migration of hematopoietic cells into and out of bone marrow is critical for normal homeostasis of lymphoid organs and of the circulating pool of leukocytes. Chemokines and adhesion molecules such as β1-integrins have been implicated in the process of mobilization of mature, hematopoietic cells from bone marrow to blood. The role of integrins in bone marrow homeostasis has been pinpointed by the observation that functional inhibition of α4β1 [very late antigen 4 (VLA-4)] integrin by antibodies elicits mobilization of hematopoietic stem/progenitor cells out of the bone marrow [27]. Neutralization of β1-integrins prevents interaction of stem/progenitor cells with endothelial and stromal cells mediated by vascular cell adhesion molecule 1 (VCAM-1), thereby favoring margination and release of stem cells to the bloodstream. VLA-4-dependent adhesion of hematopoietic stem cells to fibronectin and VCAM-1 is regulated by CXCL12/stromal-cell-derived factor-1α, suggesting that decreased responsiveness to the chemokine may play a role in the mobilization of human hematopoietic progenitors from bone marrow to blood [28]. Indeed, patients affected by WHIM syndrome present with leukopenia, despite having a hypercellular bone marrow, suggesting that mature cells are probably retained in the bone marrow because of a defect in their release into the bloodstream [29]. Myelokathexis, which is the most prominent sign of WHIM syndrome, is characterized by hypercellular bone marrow in which many hyposegmented and hypermature neutrophils can be observed, suggesting retention of these cells at the site of hematopoiesis. Diaz and co-workers [30] have recently identified heterozygous mutations of the gene encoding for CXC chemokine receptor 4 (CXCR4), the receptor for CXCL12, which result in partial truncation of the cytoplasmic tail in patients affected by WHIM syndrome. In addition, co-workers and I [31] have recently shown that neutrophils and T cell blasts derived from WHIM patients that express the same type of mutation have an increased chemotactic response to CXCL12 but a normal or slightly decreased response to CXCL8/IL-8 or CCL2/macrophage chemoattractant protein-1, as compared with cells of normal donors. CXCL8 administration to mice or to monkeys results in rapid mobilization of leukocytes and hematopoietic stem cells from bone marrow to blood [32]. Indeed, the procedure of CD34 mobilization based on G-CSF stimula-
cation elicits significant increases in serum CXCL8 levels, suggesting that the effect of G-CSF might be indirect and dependent on induction of CXCL8 [33]. Recent studies about the CXCR4 antagonist AMD-3100 have demonstrated that administration of this drug to healthy volunteers enhances the ability of G-CSF to mobilize hematopoietic cells from bone marrow. This suggests that circulating leukocyte counts depend on the homeostatic mobilization of leukocytes between bone marrow stroma and blood circulation and are regulated by the distribution and potency of chemokines in bone marrow and in circulation [34]. CXCL12 is constitutively expressed on human bone marrow endothelium and activates with α4β1-integrin and αLβ2-integrin (lymphocyte function-associated antigen 1), resulting in the arrest of circulating leukocytes and hematopoietic progenitors on vascular endothelium [35]. The heterozygous mutation of CXCR4 increases the responsiveness of mature neutrophils to its ligand in WHIM patients and augments their chemotactic response to CXCL12 expressed by bone marrow endothelium, thereby accounting for the large number of apoptotic neutrophils that are typically observed in the hypercellular bone marrow of WHIM patients. In addition, senescent neutrophils display an increased expression of CXCR4 and an enhanced response to CXLC12, which lead to their preferential homing to bone marrow, where these cells can be removed from circulation [36]. Taken together, these observations suggest that leukocytes circulate in and out of bone marrow following opposing chemokine signals coming from peripheral blood or from the site of hematopoiesis. However, the number of circulating neutrophils basically depends on their sensitivity to CXCL12 and on the amount of inflammatory cytokines that is secreted into the bloodstream during an acute-phase response (see cartoon in Fig. 1). This hypothesis is further supported by the observation that WHIM patients who are severely leukopenic despite the treatment with G-CSF can generate a surprising increase in circulating blood cells after an infectious episode (R. Badolato, personal communication). Based on these observations in WHIM patients, it can be hypothesized that the proinflammatory chemokines CXCL8, CCL2, and CCL3, which are produced in response to infectious agents, may reverse the migration of leukocytes from bone marrow to blood (Fig. 1).

The observation that leukocytes of WHIM patients have an increased chemotactic response to CXCL12 that leads to defective recirculation between intravascular and extravascular compartments prompts questions concerning the biological mechanism of this phenomenon. A possible explanation for the observed defects in leukocyte recirculation may be based on mechanism of cross-desensitization between CXCR4 and the other G-protein-coupled receptors (GPCRs). Prolonged activation of cells that express multiple GPCRs results in down-regulation of functional response of other GPCRs by a process known as heterologous desensitization. Analysis of neutrophil response to CXCL8 or to CXCL4 revealed a greater response to CXCL4 but not to CXCL8 in WHIM patients [31]. This difference in chemotactic response to CXCL4 and CXCL8 might be a result of reciprocal desensitization between CXCR4 and other chemokine receptors expressed by the same cell type. Indeed, CXCL8 can normally cross-desensitize and cross-phosphorylate CXCR4 without inducing receptor internalization by a pathway that is sensitive to staurosporine, suggesting participation of protein kinase C in this pathway [37]. Despite the imbalance in the neutrophil response to homeostatic and inflammatory chemokines, co-workers and I [31] failed to detect any abnormalities of CXCR4 internalization and calcium mobilization in response to CXCL12, except a slight delay in normalization of intracellular calcium concentration. However, cross-desensitization between GPCRs, such as CXCR4 and CXCR1, can occur without any changes in chemokine-induced calcium flux and receptor internalization, but it is partially dependent on cross-phosphorylation of the intracellular tail of

---

**Fig. 1.** (A) Round-trip trafficking amongst bone marrow, blood, and lymphoid organs. Leukocytes migrate from one compartment to another on the basis of chemokine chemotraction (represented as geometric figures) and leukocyte response to chemokines (represented as arrows). Leukocyte trafficking depends on coordinated expression of chemokine receptors along differentiation of hematopoietic precursors and chemokines between bone marrow and blood that regulate leukocyte homeostasis in blood and lymphoid organs. (B) One-way trafficking amongst bone marrow, blood, and lymphoid organs. In WHIM patients, truncating mutations of CXCR4 result in increased responsiveness of leukocytes to CXCL12, leading to abnormal retention of mature leukocytes in bone marrow. Chemokine attraction is represented as geometric figures, and leukocyte response to chemokines is represented as arrows.
the GPCR. The lack of the last 17 or 19 COOH-terminal residues of the CXCR4 tail in cells of WHIM patients may decrease the degree of phosphorylation of CXCR4, thus decreasing their sensitivity to cross-desensitization by other chemokine receptors [36]. It is likely that the degree of CXCR4 phosphorylation is already reduced in unstimulated cells of WHIM patients, thereby leading to increased responsiveness to the ligand [39]. Moreover, cross-phosphorylation and the consequent desensitization of CXCR4 in response to proinflammatory chemokines are probably reduced in neutrophils of WHIM patients, thereby leading to greater migration of cells to CXCL12 but not to other chemoattractants.

### LYMPH NODE HOMING AND HYPOGAMMAGLOBULINEMIA

The β-chemokine CXCL12 was first identified as a pre-B-stimulatory factor involved in B cell lymphopoiesis, induction of cell migration, signal transduction, and the proliferation of B cell subpopulations [40]. Consistent with this, mice deficient in CXCL12 have a severe defect in B cell development but also display an absence of bone marrow lymphopoiesis [41]. Indeed, WHIM patients also present with hypogammaglobulinemia and recurrent respiratory and skin infections, suggesting that B cell maturation and/or function could be impaired in these patients. Serum levels of all immunoglobulin (Ig) isotypes and B cell number are reduced, but distribution of B, T, and natural killer cells in peripheral blood is substantially normal [29]. In contrast, analysis of B cell precursors in the bone marrow of a WHIM patient demonstrates normal numbers of immature and mature B cells. Analysis of circulating B cells demonstrates a significant deficiency in switched memory B cells in the blood of WHIM subjects. This indicates that B cells originating from bone marrow fail to leave the site of lymphopoiesis and migrate to the secondary lymphoid organs, where they differentiate in isotype-switched B cells [31]. B and T cells of WHIM patients have normal expression of CXCR4, and T cells display an enhanced response to CXCL12, suggesting that mature B cells could be retained in the hypercellular bone marrow of WHIM patients [31]. During the B cell development, expression of chemokine receptors and the profile of chemotactic response to chemokines change dramatically. Cells of the pre-pro-B phenotype display substantial migration to CCL25 but not to CXCL13, CCL19, and CCL21. Conversely, migration in response to these chemokines was observed in pre-B and immature B cells and was even increased in naive, splenic B cells [42]. In this later stage of B cell maturation, the expression of CXCR5 and CCR7 increases, parallelizing the up-regulation of the B cell markers (e.g., CD21, CD23), which identify follicular, recirculating μ/δ+ B cells [42]. Indeed, these naive, recirculating B cells that travel between bone marrow and lymph nodes or spleen are attracted by homeostatic chemokines such as CXCL13, CCL19, and CCL21, which are expressed in lymphoid tissues [24, 43]. I have recently observed in a subgroup of patients affected by common variable immunodeficiency a selective increase in immature B cells that display reduced expression of CXCR5 and CCR7, which is typically observed in cells recently emigrated from bone marrow (Daniele Moratto, personal communication). It is likely that lower levels of CXCR5 and CCR7 detected in B cells of common variable immunodeficiency (CVI) patients are associated with reduced migration of these cells to CXCL13 and CCL21/CCL19, respectively, thereby preventing their homing to spleen or lymph nodes.

Besides neutropenia and hypogammaglobulinemia, WHIM patients display a severe lymphopenia that suggests a possible defect in the thymic output or an increased apoptosis of mature T cells. Although the original studies of CXCR4 genetic-deficient mice had ruled out a contribution of CXCL12 in early T cell development, subsequent reports have shown that CXCL12 is expressed in adult and fetal thymus and thymocyte precursors expressing CXCR4. Furthermore, these precursors functionally respond to the chemokine and have a proliferative advantage for the long-term lymphoid reconstitution of hematopoietically impaired mice [44]. Quantitative evaluation of T cell receptor excision circles (TRECS) of T cells in WHIM patients, which are considered markers of recent thymic immigrants, has demonstrated that circulating T cells have a normal number of TRECS; however, upon adjustment of their absolute number to circulating cells, have shown a reduction in the number of TRECS per milliliter of blood in WHIM patients in comparison with age-matched, healthy controls, thus suggesting that generation of T cells is still maintained but is quantitatively decreased in WHIM patients [31].

### CONCLUSION

Investigation of the pathogenesis of primary immunodeficiencies constitutes a powerful tool to reveal or define the normal mechanisms that regulate generation of hematopoietic cells from bone marrow or from thymus and their trafficking amongst blood, secondary lymphoid organs, and peripheral tissues. Some immunodeficiencies are characterized by impaired maturation of lymphocytes, which leads to progressive decline of lymphocyte number and/or Ig blood levels, as observed in WAS and CVI patients, respectively. In fact, in WAS patients, an impaired migration of T and DCs is associated with defective thymic output and with lack of DTH response to recall antigens. Moreover, in CVI patients, a defect in the normal process of up-regulation of CXCR5 and CCR7 along maturation may possibly prevent their homing to secondary lymphoid organs. All of these observations support the hypothesis that impaired lymphocyte and DC migration, which has been described in these primary immunodeficiencies, may account for the immunological defects described in WAS and CVI patients.

Other immunodeficiencies, such as WHIM, are characterized by abnormal distribution of leukocytes between blood and other compartments. The study of the mechanism at the basis of lymphopenia in WHIM patients supports the hypothesis that CXCL12 secretion in bone marrow and thymus by stromal cells is essential for the retention of polymorphonuclear neutrophils and B and T cell precursors in these organs. In physiological conditions, chemokine gradients between blood and lymphoid organs direct leukocytes to round-trip trafficking amongst bone marrow, thymus, and other organs, which results in homeo-
static regulation of leukocyte numbers and/or distribution in the blood. Conversely, a one-way circulation is observed in diseases characterized by persistent leukocytosis or leukopenia, as respectively observed in LAD and WHIM patients. Indeed, these two diseases may constitute the prototypes of defective leukocyte circulation between lymphoid compartments as a result of impaired emigration from bloodstream to peripheral tissues, as observed in LAD patients, or augmented retention of leukocytes in primary lymphoid organs, as in WHIM patients.

ACKNOWLEDGMENTS

Grants from MIUR Cofin 2002, MIUR-FIRB to Luigi D. Notarangelo, Department of Pediatrics, University of Brescia, Italy, MIUR (Centro per l’Innovazione Diagnostica e Terapeutica, IDET), PF Ministry of Health by Joint Program CNR-MIUR (Law 449/97), PS Min Salute-Bambin Gesù 2002 and Notarangelo, Department of Pediatrics, University of Brescia, Grants from MIUR Co-

REFERENCES


