

INCREASED CD34+ CELL PERIPHERAL TRAFFIC APPEARS TO BE AN UNIQUE FEATURE OF THE ALLERGIC INFLAMMATION

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SUMMARY: Evidence has been cumulated during the last years concerning the immaturity of the cells involved in the local and systemic aspects of allergic inflammation. Hematopoietic precursors (HPC) are mobilized from the bone matrix as multipotent cells or, more often, as progenitors that, after the initial white-lineage commitment reach through the peripheral blood (PB) their final destinations constituted by the target organs of allergy. Although several studies have investigated the CD34+ cells traffic and location at the level of the inflamed peripheral mucosae in allergic populations, limited information is available on their behaviour on the time-course of infectious diseases.

The current study thus was designed to assess the peripheral traffic of CD34+ HPC during the infectious inflammation. To this end CD34+ HPCs have been enumerated, by flow-cytometric techniques, in PB of 24 adult healthy beings (Group A), 24 adult subjects with symptomatic extrinsic allergy (Group B) and in PB of 24 adult patients hospitalised for febrile infectious pathology (Group C). CD34+ cell values ranged 0.01 – 0.08 % with a median of 0.03 in Group A. In Group B values ranged 0.17 – 0.75 % with a median of 0.28 and in Group C values ranged 0.00 – 0.12 % with a median of 0.07. Variance analysis test among the three groups was statistically significant ($p < 0.001$) supporting the conclusion that CD34+ HPC mobilizing and increased peripheral traffic is an unique feature of the allergic inflammation.

Key-words: Allergy, allergic inflammation - CD34+ cell - Hematopoietic precursor cell - Infectious inflammation - Infectious diseases - Mobilization - Peripheral traffic - Progenitor cell - Stem cell.

INTRODUCTION

A well established body of direct (1-13) and indirect (14-17) evidence indicates that circulating cells sustaining local and systemic allergic inflammation belong to the early stages of white lineages differentiation. Cells begin development in bone marrow and after the initial commitment move, through the blood stream, towards their peripheral final destinations.

If several experimental investigations have studied the peripheral blood (PB) traffic of the CD34+ Human Precursor (HPC) and Stem Cells (SC) during the allergic inflammation and in Healthy Human Beings, we lack information about their behaviour in infectious diseases.

The aim of the present study, thus, is to investigate whether an increased PB traffic of immature cells is also detectable during infectious inflammation or, on the contrary, it represents a peculiar feature restricted to the allergic inflammation.

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MATERIAL AND METHODS

Study populations

Peripheral CD34+ immature progenitor cells were enumerated and compared in PB of three groups of subjects. Group A (control) was of 24 subjects, 14 males and 10 females, mean age 27.8 years; all negative to the SPT allergen screening panel and negative for clinical history of allergy.

Group B (allergic) was of 24 patients, 12 males and 12 females, mean age 24.7 years, suffering from the extrinsic form (specific IgE+) of rhino-conjunctivitis (RC)

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and/or asthma (A), eczema (AD) and urticaria (Ur). Specific IgE have been investigated by routine methods that are skin prick tests (SPT) with standardised extracts of *Grasses* (Gr), *Parietaria judaica* (Pj), *Olive* (Ol), *Mugwort* (Mw), *Cupressus arizonica* (Cp), *Plantago lanceolata* (Pl), *Alternaria alternata* (Aa), *Dermatophagoides pteronyssinus* and *farinae* (Dp, Df) or the CAP-FEIA System (Pharmacia, Uppsala, Sweden) used in accordance with the manufacturer's instructions.

Group C (infectious diseases) blood samples were supplied by the Infectious Diseases Department from 24 hospitalised patients, 12 males and 12 females, mean age 54.3 years, suffering from febrile infectious pathology.

Flow cytometric enumeration of CD34⁺ cells

To investigate the whole pool of immature circulating cells, independently from the lineage commitment (Lin+/-) and the differentiation degree (CD34^{bright/dim}) we employed a simple method, based on a partial modification of the Milan Protocol (16), recently developed and elsewhere described (10).

Briefly, peripheral venous blood samples were collected in EDTA tubes; three aliquots of 50 ml of whole blood were incubated, at 4°C for 25 min. in the dark, one with 15 ml of phycoerythrin (PE)-conjugated murine monoclonal antibody (MAB) specific to CD34 molecule, one with PE-conjugated MABs specific to unrelated molecules to human leukocyte antigens, and one as unlabeled control. Third class PE anti-CD34 MAB were the Anti-HPCA2, Clone 8G12 (Becton Dickinson) or PN IM 1871 (Immunotech) suitable for routine use (17, 18). After red cell lysis, tubes were washed twice with 2 ml of cold phosphate buffered salt solution (PBS) (BD, Italy) at 1,200 revolution per minute (rpm) (300 g) for 7 min at 4°C and then resuspended in 500 ml of PBS.

Cells were analysed using a FACSCalibur with the software Cell Quest (BD) or Epics XL (IL) equipped with System II software. Three data parameters were acquired and stored in list mode files: linear forward scatter (FSC) (vertical axis), linear side-angle scatter (SSC) (horizontal axis) and log PE fluorescence, by gating whole viable cells. To enumerate CD34^{bright} and CD34^{dim} cells, the setting of the fluorescence analysis region 2 (R2) has been fixed with the lower limit at 10². For each measurement 10,000 events were acquired. Results have been expressed as percentage of cells positive.

Statistics

Data distribution within each group has been analysed by mean of *Skewness-Kurtosis* test for Normality. The distribution resulted not-normal (asymmetrical)

and thus data were expressed as median. Differences between groups were studied using the non parametric analysis of variance test of Kruskal-Wallis. A p value of 0.05 was the significance limit.

RESULTS

CD34⁺ cell values in healthy subjects (Group A) ranged from 0.01 % to 0.08 %. The median was 0.03 %.

In allergic patients, (Group B), CD34⁺ values ranged from 0.17 % to 0.75 % with 0.28 % as median.

In patients with infectious diseases (Group C) CD34⁺ cells values ranged from 0.00 % to 0.12 %. The median was 0.07 %.

Median values of the three groups resulted significantly different ($H = 55.48$; $p < 0.001$).

Data have been resumed in fig. 1.

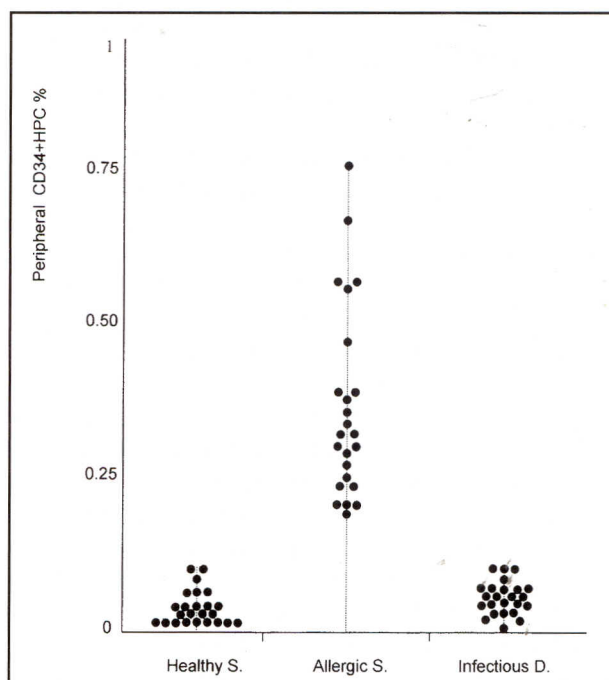


Figure 1: Values of circulating CD34⁺ Haemopoietic Precursor Cells in peripheral blood of healthy subjects (Group A) (median 0.03), allergic symptomatic subjects (Group B) (median 0.28) and in subjects with febrile infectious diseases (Group C) (median 0.07). Median values in Groups A and C were significantly ($p < 0.001$) lower than Group B.

DISCUSSION

In adult healthy human beings the haematopoietic precursor cells (HPC) reside within the bone marrow matrix and only an insignificant amount could be detectable in peripheral blood. On the other hand, during allergic inflammation, highly immature multipotent uncommitted precursors (CD34^{bright} CD33⁻ CD38⁻) move in PB of patients with high degrees of

symptoms severity as well as less undifferentiated, lineage-committed progenitors (CD34^{dim}), circulate when inflammation is low (10).

If above mentioned confluent lines of evidence have raised the emerging systemic and dynamic common model for the pathogenesis of allergic disease, built on the concept of an impaired peripheral differentiation of myeloid and lymphoid lineages (21, 22), the involvement potential of HPC in the infectious inflammation has not been investigated.

Data of the present study show that during febrile infectious inflammation CD34+ HPCs are not significantly mobilized. Median value of 0.07, in fact, is below the 0.10 values previously established as the upper limit of the normal range (10) and is significantly lower with respect to the allergic group ($p < 0.001$). Group C subjects, furthermore, have been randomly enrolled on the single criterion of suffering from infectious disease, independently from being atopic or not. Results add further evidence to unravel the complex mechanisms underlying allergic disease, particularly on the concept of a compensatory "leukocytopoiesis extra demand".

Relationship existing between stress and the development of allergic symptoms is a widely held notion clarified by the acquisitions that chronic stress suppresses cellular immunity and induces a TH2/TH1 unbalance (23-26). Lymphocyte precursors are highly susceptible to activate apoptosis-programs when exposed to glucocorticoids (27-29). Stress conditions such as exercise, pregnancy and puerperium are thus capable of inducing a severe lymphocyte depletion that, being the immune system redundant and compensatory, may activate a lymphopoiesis extra-demand.

HPC mobilization from marrow microenvironment and the subsequent traffic in peripheral blood involve complex mechanisms still under investigation. Several factors have been identified to be involved in mobilization, including chemokines, cytokines, adhesion molecules and stromal cells (30) as well as chemo-radiotherapeutic immunosuppressant regimens. Immunosuppressors, in fact, are routinely employed to induce or to enhance mobilization for peripheral blood stem cells harvesting (31-34). A recent report, furthermore, directly links suppressant conditioning regimen with the development of airway hyperreactivity (35) that is a feature of allergic respiratory diseases.

Mucosal hyperreactivity is not confined to the airways; in atopic dermatitis patients with overt symptoms limited to the skin, in fact, the existence of respiratory and gut mucosal hyperreactivity has been reported (36-38), supporting the view that non-specific hyperreactivity depends on the systemic cellular mechanism underlying the allergic inflammation and involves all the mucosae (10).

Data of the present investigation demonstrate that the

peripheral traffic of CD34+ progenitor and stem cells in allergic subjects is significantly ($p < 0.001$) increased when compared with healthy beings and subjects suffering from infectious diseases and thus support the conclusion that CD34+ HPC mobilizing and circulating are a unique feature of the allergic inflammation. Results add further reliable information for a better understanding of the complex mechanisms involved in the pathogenesis of the allergic diseases.

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