

# QUANTITATIVE EXPRESSION OF THE MAJOR HOMING INTEGRINS $\alpha$ L AND $\alpha$ 4 ON HUMAN CORD BLOOD HEMATOPOIETIC PROGENITOR AND STEM CELLS

F. Mastrandrea<sup>(1)</sup>, G. Coradduzza<sup>(2)</sup>, L. De Vita<sup>(3)</sup>,  
G. Serio<sup>(4)</sup>, A. Minardi<sup>(1)</sup>, M. Manelli<sup>(2)</sup>, F. Pezzuto<sup>(5)</sup>, L. Muratore<sup>(6)</sup>

**SUMMARY:** An increased traffic of hematopoietic progenitor cells (HPC) between bone marrow and peripheral organs is a peculiar feature of the allergic inflammation. It has been recently reported that the sublingual form of specific immunotherapy (SLIT) is capable of reducing such an increased HPC traffic.

The House Dust Mite major antigen Der p1 has been proved to up-regulate the expression of the ICAM-1 and VCAM-1 endothelial addressins, supporting the view of an inflammatory cell recruiting at the site of allergen extract administration. In the present work we have investigated, by flow-cytometric techniques, the expression of the two major integrins CD11a (LFA-1) and CD49d (VLA-4) that are the homing receptor cognate for ICAM-1 and VCAM-1 on human cord blood CD34 hematopoietic progenitor and stem cells. Even if both the investigated molecules resulted detectable on CD34+ HPC surfaces, being the system redundant, the density of the cellular expression was significantly higher for CD49d (median value: 158) than CD11a (median value: 20.5), suggesting a preferential usage of the homing axis VLA-4/VCAM-1. Results consistency with outcomes of clinical trials that relate SLIT efficacy to allergen dosage is discussed.

**Key-words:** Allergy - CD34, Der p 1 - Hemopoietic precursor cell - ICAM-1 - LFA-1 - Sublingual immunotherapy - SLIT - VCAM-1 - VLA-4.

## INTRODUCTION

Hematopoietic progenitor and stem cells contribute to the allergic inflammation, both in systemic and local aspects (1-8) and their traffic is regulated mainly by chemokines and adhesion molecules (CAMs). It has been recently described that House Dust Mite allergens, particularly Der p 1, are capable of up-modulating the endothelial expression of the addressins ICAM-1 and VCAM-1 (9), through the activation of the NF- $\kappa$ B factor (10-12), supporting the view that at least part of the mucosal route of administration of specific immunotherapy effects may derive from recruiting of inflammatory cells at the site of allergen release (9, 13, 14). To further understand such a mechanism, among the redundant repertoire of homing receptors, in the present study we have investigated the physiological expression of

the counterligands specific for ICAM-1 and VCAM-1 on human umbilical cord blood CD34 hematopoietic progenitor and stem cells.

## MATERIAL AND METHODS

Twenty-two unselected Cord Blood venous samples were supplied by the Neonatology Department from full-term deliveries after informed consent and in accordance with the procedures approved by the local independent ethic committee (Allergy and Stem cells Study).

The Milan Protocol of Peripheral Blood CD34+ Cell Estimation (15) was followed with partial modification elsewhere described (8). Briefly, cord blood venous samples were collected in ethylenediaminetetra-acetic acid (EDTA) tubes. Three aliquots of 100  $\mu$ l of whole blood were incubated, at 4°C for 25 min. in the dark, as follows: one tube contained 10  $\mu$ l of phycoerythrin (PE)-conjugated murine monoclonal antibody (MAB) specific to CD34 molecule and 10  $\mu$ l of fluorescein (FITC)-conjugated MAB specific to CD11a. One tube contained 10  $\mu$ l of (FITC)-conjugated MAB specific to CD34 molecule and 10 ml of PE-conjugated MAB specific to CD49d. One tube served as unlabeled control.

Monoclonal antibodies were from Becton Dickinson (BD), Milan, Italy. PE and FITC anti-CD34 MABs were

<sup>(1)</sup> Allergy and Clinical Immunology O. U., SS Annunziata Hospital, AUSL TA1, Taranto (Italy).

<sup>(2)</sup> Allergy and Cellular Immunology Lab., SS Annunziata Hospital, AUSL TA1, Taranto (Italy).

<sup>(3)</sup> Division of Neonatology, SS Annunziata Hospital, AUSL TA1, Taranto (Italy).

<sup>(4)</sup> Medical Statistics, Public and Internal Medicine Dpt., University of Bari, Bari (Italy).

<sup>(5)</sup> Allergy and Clinical Immunology O. U., AUSL SA2 (Italy).

<sup>(6)</sup> Allergy and Clinical Immunology O. U., Vito Fazzi Hospital, AUSL LE1, Lecce (Italy).

the Anti-HPCA2, Clone 8G12; FITC anti-CD11a was the Anti-Human LFA-1 $\alpha$ , Clone G-25.2 and PE anti-CD49d was the Anti-VLA- $\alpha$ 4, all from BD.

After incubation 2 ml of red blood cell lysis buffer (BD, Italy) were added to each tube and incubated at room temperature for 10 min, in the dark. After lysis cells 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  $\mu$ l of PBS.

Cells were analysed using a FACScan flow cytometer equipped with Lysis 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 or FITC fluorescence, by gating whole viable cells. At least 10,000 events were acquired for each measurement. Double labelled cells (CD34/CD11a) and (CD34/CD49d) were expressed as percentage of positive cells (Dot Plot analysis). The quantitative expression of the CD11a and CD49d molecules was evaluated and reported analysing the mean fluorescence density (Histograms) in the CD34+ region.

## RESULTS

The two homing molecules investigated were both qualitatively expressed on the majority of the CD34 cells (CD34+/CD11a+ median value: 97; CD34+/CD49d+ median value: 98). The density of the CD34 HPC expression was more than seven times higher for CD49d (median value: 158) with respect to CD11a (median value: 20.5). Data source is reported in Tab. I.

## DISCUSSION

Whether in healthy human beings the majority of hematopoietic progenitor cells (HPC) resides within the bone marrow and only a negligible number recirculates in peripheral blood (PB), an increased cell traffic between bone marrow and peripheral organs constitutes a peculiar feature of the allergic inflammation (7, 8, 16-19).

HPC mobilization from marrow microenvironment into circulation and the subsequent localization in target tissues is a multistep complex mechanism not yet fully understood. After the initial model based on the

**Table 1:** CD34+ cord blood cells positive for the two major homing integrins LFA-1 $\alpha$  CD11a and VLA- $\alpha$ 4 CD49d and cellular quantitative expression of the two molecules.

| Sample | CD34+/CD11a+% | CD34+/CD49d+% | CD11a<br>Fluor.density | CD49d<br>Fluor.density |
|--------|---------------|---------------|------------------------|------------------------|
| 1      | 100           | 100           | 22                     | 173                    |
| 2      | 95            | 100           | 16                     | 131                    |
| 3      | 100           | 100           | 11                     | 96                     |
| 4      | 100           | 100           | 25                     | 112                    |
| 5      | 100           | 100           | 22                     | 134                    |
| 6      | 75            | 100           | 10                     | 106                    |
| 7      | 93            | 100           | 19                     | 104                    |
| 8      | 97            | 100           | 18                     | 134                    |
| 9      | 100           | 96            | 12                     | 165                    |
| 10     | 90            | 99            | 25                     | 180                    |
| 11     | 100           | 94            | 15                     | 163                    |
| 12     | 97            | 100           | 26                     | 156                    |
| 13     | 96            | 97            | 21                     | 131                    |
| 14     | 100           | 86            | 22                     | 211                    |
| 15     | 80            | 100           | 8                      | 125                    |
| 16     | 98            | 96            | 17                     | 160                    |
| 17     | 97            | 97            | 33                     | 202                    |
| 18     | 94            | 96            | 20                     | 172                    |
| 19     | 96            | 87            | 39                     | 179                    |
| 20     | 96            | 89            | 34                     | 181                    |
| 21     | 100           | 92            | 16                     | 168                    |
| 22     | 100           | 88            | 26                     | 67                     |
|        | Median v. 97  | Median v. 98  | Median v. 20.5         | Median v. 158          |

administration of repetitive stimulations with Granulocyte-Colony Stimulating Factor (G-CSF) alone or in combination with immunosuppressants like cyclophosphamide (20), the focus has moved to the identification of major roles played by the chemokine stromal-cell derived factor 1 (SDF-1) and adhesion-homing related molecules.

SDF-1 is a phylogenetically ancient chemokine constitutionally expressed in a variety of tissues and thus regarded as a physiological axis governing, with the receptor CXCR4 expressed on CD34<sup>+</sup> HPC and on TH2 lymphocytes, cells recirculation (21-25). SDF-1/CXCR4 have been recently involved also in allergic airway inflammation (26, 27) as well as in remodelling (28).

Transendothelial migration of CD34<sup>+</sup> progenitor and stem cells is mediated mainly by beta-integrins and PECAM-1 (CD31) rather than E or L-selectin (29, 30).

Data from the present investigation confirm that the two major integrins CD11a (LFA-1) and CD49d (VLA-4) are both expressed on cord hematopoietic precursors because the system is redundant.

Quantitative analysis, nevertheless, showed a higher membrane expression of the latter than the former, suggesting a preferential usage of such a homing molecule, within the system redundancy.

The recent better definition of the physiological model for HPC and stem cells homing in NOD SCID mice (23) and in humans (31) indicates an inverse correlation between maturity and homing efficiency.

During allergic inflammation a significant traffic of highly immature multipotent uncommitted precursors (CD34<sup>bright</sup> CD33<sup>-</sup> CD38<sup>-</sup>) is detectable only in patients with severe symptoms; on the contrary, when symptoms are low-to-moderate, that is the conditions required to start specific immunotherapy, less undifferentiated lineage-committed cells (CD34<sup>dim</sup>) move. A less efficient homing of the CD34<sup>-</sup> HPC contributing to the allergic inflammation could be an important factor for a better understanding of drug and immunotherapeutic medical interventions.

It has been well defined that, e. g., the major mite allergen Der p 1 is capable of activating the nuclear factor  $\kappa$ B (NF- $\kappa$ B), that is one of the pathways for the adhesion molecules transcription (10, 11). Der p 1 in fact up-modulates the addressin ICAM-1, weakly but constitutionally expressed on the resting vessels endothelium and it also induces the "de novo" synthesis of the addressin VCAM-1, not expressed on human endothelium in baseline conditions (9).

The significant major expression on the CD34<sup>+</sup> HPCs of the CD49d, that is the homing cognate receptor for the vascular addressin VCAM-1, whose expression is *de novo* induced by allergen stimulation, suggest for a major role of the axis CD49d VCAM-1 in the investigated mechanism potential of mucosal recruitment of the CD34<sup>+</sup> cells induced by the local form of specific immunotherapy like the sublingual (SLIT). Since VCAM-1, differently from ICAM-1, is not constitutionally expressed on vessels endothelium, it is conceivable that to obtain an effective steady-state over-expression of such an addressin leading to the preferential recruitment of the inflammatory immature circulating cells at the site of the allergen extract administration, the allergen amounts should be high enough. Our results are thus consistent with outcomes of clinical trials supporting a major efficacy of high-dose SLIT (32).

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## MENACING MOLDS: ARE THEY AFFECTING YOUR ALLERGIES?

**MILWAUKEE** - April showers can increase the amount of mold found inside and outside the home. Along with pollens from trees, grasses, and weeds, molds are an important trigger of allergic rhinitis, affecting nearly 40 million Americans, according to the American Academy of Allergy, Asthma & Immunology (AAAAI).

"Mold spores contain allergens, substances that some immune systems recognize as dangerous," said Robert K. Bush, MD, Fellow of the AAAAI and member of the AAAAI's Indoor Allergen Committee. "Exposure to mold can trigger an allergic reaction such as nasal stuffiness, eye irritation, wheezing, coughing and hay fever-like symptoms."

Molds are microscopic organisms that are pervasive in any outdoor environment. However, they may be brought inside via clothing, shoes and soils. Mold needs only a food source, warm environment and moisture to grow. Mold may be invisible to the eye or may look like furry growth, black stains or specks of black, white, orange, green and brown on surfaces. Large infestations of mold can usually be seen or smelled.

### ■ Where can mold be found?

#### ➤ Inside:

- Basements or crawl spaces
- Near windows
- Under sinks
- Near leaky pipes

#### ➤ Outside:

- Near heavy vegetation
- Near hay or straw
- In piles of leaves
- In grass

### ■ What can be done?

The AAAAI recommends that you take the following steps to rid your home of mold:

- Repair leaking roof and pipes ;
- Maintain a low level of humidity in your home (between 35-40 percent); if necessary, use a de-humidifier to remove moisture ;
- Use a solution of warm water, liquid detergent and 5% bleach to clean the mold on washable surfaces. Use exhaust fans in the bathroom and wipe down the shower after use.

If mold is visible on soft spaces, such as carpeting or wallpaper, these things should be removed.