

From bone marrow to airways in allergen-induced airway inflammation

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Göteborgs Universitet
kommer att offentligen försvaras i föreläsningssal Herman Krefting 1, Bruna Stråket 11B,
Sahlgrenska Universitetssjukhuset, Göteborg

Fredagen den 19 mars 2004, kl. 09.00

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- I. A-K Johansson, S Sergejeva, M Sjöstrand, JJ Lee and J Lötvall.
Allergen-induced traffic of bone marrow eosinophils, neutrophils and lymphocytes to airways.
Submitted for publication
- II. B Sitkauskiene, A-K Johansson, S Sergejeva, S Lundin, M Sjöstrand and J Lötvall.
Regulation of bone marrow and airway CD34⁺ eosinophils by IL-5
American Journal of Respiratory Cell and Molecular Biology 2003; published online as First Edition paper
- III. A-K Johansson, M Sjöstrand, M Tomaki, A-M Samuelsson and J Lötvall.
Allergen stimulates bone marrow CD34⁺ cells to release IL-5 *in vitro*; a mechanism involved in eosinophilic inflammation?
Submitted for publication
- IV. A-K Johansson, M Sjöstrand, C Malmhäll, M McKinnon and J Lötvall.
Human blood and bone marrow CD34⁺ cells produce IL-5; autocrine regulation of eosinophilopoiesis?
Submitted for publication

Göteborg 2004

From bone marrow to airways in allergen-induced airway inflammation

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The airway (AW) inflammation in asthma includes eosinophils, neutrophils and T-lymphocytes. The accumulation of inflammatory cells within the AW is considered to be a result of increased production of inflammatory cells within the bone marrow (BM), a release into the circulation and subsequent migration into the AW but so far there is limited evidence that BM cells have the capacity to migrate into the AW in allergen-induced AW inflammation. Eosinophils develop from CD34⁺ progenitor cells under the influence of IL-5, IL-3 and GM-CSF. Recent studies have shown increased numbers of these CD34⁺ progenitor cells in BM and AW of allergic subjects.

The aims of this thesis were to; A) evaluate whether BM-derived inflammatory cells have capacity to traffic into the AW in response to allergen exposure, B) determine the effects of allergen exposure and IL-5 in the AW and BM inflammatory response, including CD34⁺ cells and C) evaluate mechanisms involved in the regulation of BM eosinophilopoiesis. To assess this, we used mouse models of allergen-induced AW inflammation. Cells produced during the allergen exposure period were identified using a thymidine analogue, bromodeoxyuridine (BrdU). BM or peripheral blood (PB) derived-CD34⁺ cells from either mouse or human were stimulated *in vitro* to determine whether these cells can release eosinophilopoietic cytokines.

Adoptive transfer of BM inflammatory cells (BrdU⁺ cells) showed traffic of not only eosinophils but also of neutrophils and lymphocytes into the AW in response to an AW allergen exposure. Allergen exposure of sensitized wild type (C57BL/6) and mice overexpressing IL-5 specifically in their CD3 cells (NJ.1638) increased the number of BrdU⁺ eosinophils, neutrophils, lymphocytes and CD34⁺ cells in AW. Furthermore, it increased the relative number of eosinophils in BM. Overexpression of IL-5 further enhanced the AW and BM eosinophilic response.

Systemic treatment with an anti-IL-5 antibody (TRFK-5) in allergen-exposed mice reduced the relative number of BrdU⁺ and CD34⁺ eosinophils in the BM three days after the administration. In broncho- alveolar lavage fluid (BALf) the reduction was most clear five days after the administration.

Adoptive transfer of IL-5 overproducing CD3⁺ splenocytes to sensitized wild type mice caused enhanced relative number of BM eosinophils. Further investigations by transferring CD4⁺ and CD8⁺ T-lymphocyte subsets from either wild type or mice overexpressing IL-5 to immunodeficient mice (SCID-bg) suggested that the BM eosinophilia at least partly is regulated by CD8⁺ lymphocytes, although IL-5 enhances the response.

Allergen stimulation of mouse BM CD34⁺ cells caused release of IL-5, but not IL-3 and GM-CSF *in vitro*. However, un-specific stimulation induced release of all three cytokines. An IL-5R α -antagonist (E12K) reduced the maturation of PB CD34⁺ cells to eosinophils *in vitro*.

In conclusion, this study argues that inflammatory cells that have been produced in the BM, and are released into the blood, traffic to the AW following airway allergen exposure. IL-5 plays a central role in allergen-induced AW inflammation and substantially contributes to the enhanced BM eosinophilopoiesis. The BM eosinophilopoiesis is at least partly regulated by CD8⁺ lymphocytes although additional mechanisms may occur. One such possible mechanism could be that the CD34⁺ cells themselves respond to allergen exposure and thereby may act in an autocrine way, enhancing the eosinophilic response.

Keywords: bone marrow, allergic airway inflammation, CD34⁺ cell, CD8⁺T-lymphocytes, eosinophils, eosinophilopoiesis, neutrophils, lymphocytes, IL-5, IL-3, GM-CSF, TRFK-5, IL-5R α

ISBN:91-628-5958-7