Regulation of IL5R on eosinophil progenitors in allergic airway inflammation


J. A. DENBURG

Department of Medicine, McMaster University, Hamilton, Ontario, Canada

Introduction

Recent studies of the involvement of the bone marrow in human atopic asthmatic responses to inhaled allergen confirm what we have found in a canine model of Ascaris suum-induced bronchial hyperresponsiveness. CD34/45⁺ hemopoietic progenitors, increased in numbers in the blood and marrow of atopic individuals, can be specifically upregulated following airway allergen challenge eliciting bronchial hyperresponsiveness and the late-phase response. All the IL-5 responsive subset of progenitors, making the Eo-B lineage specifically, is upregulated in the marrow within 24 h of allergen challenge in dual responder asthmatics. Using triple colour flow cytometry, it can be shown that this subpopulation of progenitors in the marrow is one that bears high affinity receptors for IL-5 (IL-5Rz), existing as a subpopulation of early progenitors bearing CD34/45 (1,2). Thus, the readily mobilizable pool of autocrine [GM-CSF- and IL-5-producing (3)] Eo-B progenitors at a very early stage of lineage commitment is increased after inhalation of allergen, only in those individuals who develop ongoing inflammatory responses. The nature of the signalling between the airway and the bone marrow, which upregulates IL-5Rz on CD34⁺ progenitors in the bone marrow in vivo, is not yet known. We therefore undertook studies to explore the in vitro regulation of IL-5R expression on hemopoietic progenitors.

Effects of retinoic acid

IL-5 plays a central role in eosinophil and basophil differentiation, exerting its effects through the IL-5 receptor. Though the z chain of the IL-5R is known to exist as either a membrane-bound or soluble isoform, little is currently known concerning regulation of IL-5Rz gene transcription in the context of commitment of hemopoietic progenitor cells to the eosinophil and basophil lineages.

Recent studies by Tavernier et al. have indicated that IL-5 itself can regulate IL-5Rz expression on cord blood-derived mature eosinophils; recent studies in our laboratory indicate that the same holds for bone marrow eosinophil progenitors. Given that all-trans retionid acid (ATRA) is known to modulate some aspects of haemopoietic differentiation, we examined the effects of ATRA on eosinophil/basophil differentiation and IL-5Rz expression. In semi-solid cultures of normal human bone marrow, ATRA selectivity suppressed eosinophil/basophil differentiation of cord blood CD34⁺ cells, while neutrophil differentiation proceeded without impediment. Most importantly, these effects of ATRA or CD34⁺ cells were associated with selective, dose dependent inhibition of membrane-bound IL-5Rz, upregulation of soluble IL-5Rz transcription, but no change in GM-CSF receptor expression. These findings indicate that retinoids can differentially regulate membrane and soluble isoforms of IL-5Rz, and that these effects have functional consequences in vitro on eosinophil and basophil differentiation.

Cord blood studies: prediction of atopy?

The above findings point to an association between allergic asthma and increased responsiveness of myeloid progenitor cells to certain haemopoietic growth factors. However, it is not clear at what age these changes in progenitor cells first becomes manifest, though increasing evidence suggests that the allergic phenotype may begin to emerge in very early life. We therefore compared expression of haemopoietic cytokine receptors on CD34⁺ progenitor cells in cord blood from normal infants (at ‘low risk’ for subsequent atopy), and infants with at least one atopic first degree relative (at risk’ for subsequent atopy), by flow cytometry. Although no differences in absolute CD34⁺ numbers were observed between the two groups, expression of GM-CSF receptor on CD34⁺ cells was significantly reduced in the ‘at risk’ compared to the ‘low risk’ group (P=0.021), with a tendency to reduced IL-3 and IL-5 receptor expression in the ‘at risk’ group (4). While the functional sequelae of reduced GM-CSF receptor expression on CD34⁺ cells remain to be determined, these findings show an association between genetic risk for atopy and changes in the expression of haemopoietic cytokine receptors on cord blood progenitor cells.

References


IL-5Rα is upregulated by IL-5 and IL-9 during eosinophil development, and CD34/IL-5Rα cells can be detected in the airway in asthma

D. S. ROBINSON, S. M. RANKIN, B. GREGORY, Q. HAMID, A. B. KAY AND J. TAVERNIER

Allergy and Clinical Immunology, Imperial College School of Medicine at the National Heart and Lung Institute, Leukocyte Biology, Biomedical Sciences Division, Imperial College School of Medicine, Meakins Christie Laboratories, Montreal, Canada

Inter University Institute for Biotechnology, University of Ghent, Belgium

Allergy and Clinical Immunology, Imperial College School of Medicine at the National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, U.K.

Interleukin-5, but not IL-3 or GM-CSF, upregulate IL-5Rα expression

The receptor for interleukin 5 receptor consists of a cytokine-specific α chain (IL-5Rα), and a signalling β chain, which is shared with IL-3 and GM-CSF (1,2). These three cytokines can act in eosinophil development and activation in vivo, but gene deletion or antibody blocking of IL-5 largely ablates eosinophilic responses in models of allergic disease or helminth infection. Differential IL-5Rα gene splicing generates either the membrane-anchored isoform (TM-IL-5Rα), which associates with the common β chain to allow IL-5 responsiveness, or a secreted, antagonist variant (SOL-IL-5Rα) (3). To examine the expression of IL-5Rα isoforms during human eosinophil development a system of in vivo eosinophil development from umbilical cord blood CD34+ progenitor cells grown in IL-3 and IL-5 was used. These cells developed towards the eosinophil lineage with 84-5%, (range 75–88%, n = 10) expressing major basic protein and showing characteristic eosinophilic granules by day 14. Competitive RT-PCR showed upregulation of IL-5Rα-TM mRNA relative to that of the soluble isoform at day 5–7 of culture in IL-3+IL-5. IL-5Rα expression at the cell surface also increased over time in culture and a maximal chemokinetic response to IL-5 was seen by day 7 of culture in IL-3+IL-5. This upregulation of TM-IL-5Rα was also seen in eosinophils developing in IL-3 or IL-3 and GM-CSF without exogenous IL-5. However, the switch to TM-IL-5Rα appeared to be IL-5 dependent, since eosinophil development was blocked by inclusion of anti-IL-5 (antibody 5A5) in cultures, and IL-5 mRNA was demonstrated at day 3–5 before TM-IL-5Rα upregulation. Similar switching from SOL-IL-5Rα to TM-IL-5Rα was shown in a mingene system in transfected cell lines, and such switching was seen in response to IL-5, but not IL-3 or GM-CSF.

These findings suggest that eosinophil development and lineage expansion is IL-5 dependent, in keeping with the pronounced eosinophilia seen in IL-5 transgenic mice (4), and raise the possibility of IL-5 specific signalling (not shared by IL-3 and GM-CSF, which with IL-5 have a common receptor β subunit).

Interleukin-9 increases eosinophil expansion through upregulation of IL-5Rα

IL-9 has been linked by genetic studies to bronchial hyperresponsiveness (BHR) in mice and humans, and transgenic targeting of IL-9 to the airway epithelium in mice leads to eosinophil infiltration, mast cell hyperplasia and BHR (5,6). Addition of IL-9 to human umbilical cord blood CD34+ cells cultured in IL-3 and IL-5 increased cell yields at day 7 and day 14 in a dose dependent manner, (mean increase in cell number at day 7, 17%, range 11–23%, n = 5, and 35% at day 14, range 13–57%, n = 5). In addition, flow cytometry showed that addition of IL-9 to CD34+ progenitors grown in IL-3 and IL-5 increased expression of IL-5Rα at day 7 and 14, mean increase in fluorescence for IL-9 added to IL-3+IL-5 at day 7, 47% n = 3, and 80-4% at day 14 n = 3. RT-PCR showed that mRNA for both soluble and membrane associated IL-5Rα isoforms was induced at days 7 and 14 of culture in IL-9 alone. In addition, IL-9 increased IL-5Rα expression by mature eosinophils and HL-60 cells developing towards the eosinophil lineage (not shown). These data suggest that one mechanism by which IL-9 may contribute to BHR is by increased IL-5 responsiveness of developing and mature eosinophils.

CD34+/IL-5Rα mRNA+ cells can be detected in the airway in asthma

CD34+/IL-5Rα mRNA+ cell numbers detected by simultaneous immunohistochemistry for CD34 and in situ hybridization were increased in bronchial biopsies from asthmatic subjects when compared to atopic non-asthmatics and non-atopic control subjects (7). Amongst the asthmatic subjects there was a significant correlation between eosinophil numbers and numbers of IL-5Rα mRNA+ cells in bronchial biopsies (rS = 0.90, P < 0.001) and between FEV1 and CD34+/IL-5Rα mRNA+ cell numbers for asthmatic subjects (rS = –0.72, P < 0.02)