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Cytokines and allergy

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The survival and state of activation of eosinophils and basophils depend on peptide growth factors or cytokines. Many of the cytokines that activate the function of mature cells also stimulate the proliferation and differentiation of their precursors and thus act in a different manner along the whole lineage.

FUNCTION OF CYTOKINES

Cytokines act on eosinophils and basophils by binding to specific receptors. In the case of hemopoietic growth factors (including interleukin [IL]-3, granulocyte-macrophage colony-stimulating factor [GM-CSF], IL-5) the receptors belong to the cytokine receptor superfamily. These are either heterodimers or homodimers, but the latter appear to act as functional heterodimers because the same chain binds different parts of a ligand and interacts asymmetrically. In each instance

Abbreviations used

GM-CSF: Granulocyte-macrophage colony-stimulating factor
IL: Interleukin
MAb: Monoclonal antibody
R α : Alpha-chain receptor
TNF: Tumor necrosis factor

there appears to be a specific binding of the ligand to one of the chains of the homodimer or the α chain of the heterodimer receptor and then to the second or the β chain, conferring a higher affinity of binding and signaling capacity to the complex (Table I).

Hemic cells typically display a small number of high-affinity receptors (usually a few hundred) of which occupancy of 10% to 20% is sufficient for signaling. Because IL-3, GM-CSF, and IL-5 share a common β chain, there is no additive activation by a combination of these cytokines. However, typically, strong synergistic effects are seen with cytokines of different receptor families, which act in concert on a given cell type.

Regulation of cytokine production

An important level of control of cytokine effects is at the level of production. Cells have a specific repertoire of cytokines that can be produced (Table II), and because most act in a

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TABLE I. Approximate binding affinities of hemopoietic growth factors

Receptors*	IL-3	GM-CSF	IL-5
α IL-3	10^{-7} - 10^{-8} mol/L	—	—
α GM-CSF	—	10^{-9} mol/L	—
α IL-5	—	—	10^{-10} mol/L
α IL-3 + β	10^{-11} mol/L	—	—
α GM + β	—	10^{-11} mol/L	—
α IL-5 + β	—	—	10^{-11} mol/L

*Greek letter α refers to the specific α chain for each hemopoietic growth factor, and β refers to the common signaling chain.

TABLE II. Cellular sources of hemopoietic growth factors

Growth factor	Cellular source
IL-3	T lymphocytes, mast cells (?)
GM-CSF	T lymphocytes, mesenchymal cells, hemic cells
IL-5	T lymphocytes

TABLE III. Activation of GM-CSF gene expression in endothelial cells

Treatment	GM-CSF expression (%)
Nil	10
PMA, A23187	100
PMA, A23187, CsA	31

A23187, Calcium ionophore A23187, 2 μ mol/L; CsA, cyclosporine, 0.1 μ mol/L; PMA, phorbol myristate acetate, 20 ng/ml.

paracrine fashion in a given microenvironment, the type of cells present are a major determinant.

Activated T lymphocytes are a rich source of IL-3, IL-5, and GM-CSF, as well as of IL-4 and interferon- γ . Interestingly, most of the genes for these cytokines are found on the long arm of chromosome 5 in a region spanning less than 1 megabase. The production of GM-CSF and IL-3 appears to be regulated by a common intergenic enhancer and is inhibited by cyclosporine acting through this enhancer.¹

It has been known clinically that inflammatory and especially allergic diseases respond readily to steroids and cyclosporine, and it is possible that a major part of that response is due to inhibition of cytokine production.

Table II indicates that GM-CSF is also produced by cells other than T lymphocytes. However, cytokine production has not been known to be cyclosporine sensitive in cells other than T lymphocytes, other than production of IL-3, GM-

CSF, and IL-8 by eosinophils stimulated by phorbol myristate acetate and ionophore.² Thus we decided to investigate whether GM-CSF production in endothelial cells is regulated by cyclosporine. The striking observation was that the inducible expression of GM-CSF in endothelial cells was also repressible by cyclosporine (Table III), and this activation of GM-CSF gene expression appeared to be mediated by the enhancer previously mentioned.⁶

Receptor expression

Little is known about the regulation or function of cytokine receptor expression. Debate abounds whether control of receptor expression is important. On the one hand, the low occupancy needed for full biologic action suggests that small changes in the level of expression would not be noticed. On the other hand, receptors are often shed and their continuous resynthesis is necessary for steady expression.

Given the paucity of receptors, until recently it has been difficult readily to diagnose their expression. Recently, Sun and Simmons of Prof. Lopez's laboratory have made a series of monoclonal antibodies (MAb) that detect the expression of IL-3 α -chain receptor (R α), GM-CSF-R α , and the common β chain on eosinophils (Fig. 1). Studies by these scientists and by Smith and van den Heuvel are under way in an attempt to correlate the expression of these receptors with clinical states. Interesting trends are already being observed in experiments in which eosinophils are incubated for several days with hemopoietic growth factors and receptor expression is measured by flow cytometry. Fig. 1 shows that incubation with GM-CSF results in down-regulation of its own α chain but, interestingly, strong upregulation of IL-3R α and to a lesser extent the common β chain.

Role of IL-3 in endothelial cell function

A surprising discovery recently made in our laboratory by Korpelainen et al.³ concerned the

TABLE IV. Eosinophil transmigration on HUVEC monolayer

Endothelial stimulation	Eosinophil preincubation	
	HUVEC medium overnight	GM-CSF (100 pmol/L) overnight
Nil	4.1 ± 1.3*	3.0 ± 0.4
TNF (100 U/ml, 6 hr)	3.2	3.4 ± 0.5
IL-3 (30 µg/ml, 6 hr)	3.1 ± 0.2	3.4 ± 0.0
TNF + IL-3	3.1 ± 0.1	3.5 ± 1.3
IL-4 (1 ng/ml, 6 hr)	4.1 ± 0.3	7.8 ± 1.5
IL-3 + IL-4	9.7 ± 4.4	10.7 ± 0.8

HUVEC, Human umbilical vein endothelial cell; U, units.

*Percentage of transmigrated eosinophils (No. of Cells in Lower Well ÷ No. of Cells Added in Upper Well) after 2 hours, expressed as mean ± SEM.

TABLE V. Inhibition of IL-3 binding and function by MAb 7G3

	No MAb	1 µg/ml 7G3
Binding of ¹²⁵ I-labeled IL-3 + IL-3Rα transfectants (%)	100	2
Stimulation of basophil degranulation by IL-3* (% histamine release)	100	13

*Basophil degranulation was stimulated by anti-IgE added to IL-3-incubated blood basophils.

TABLE VI. Inhibition of GM-CSF function by GM-Arg²¹

	No antagonist	GM-Arg ²¹ , 100 ng/ml
Binding of ¹²⁵ I-labeled GM-CSF to GM-CSFR α-chain transfectants	100%	33%
Neutrophil O ₂ production*	14 nmols/min/10 ⁶ cells	6.8 nmols/min/10 ⁶ cells

GM-Arg²¹, GM-CSF derivative, with arginine substituted for glutamic acid in position 21.

*GM-CSF was used to prime neutrophils for O₂⁻ generation, and 10⁻⁸ mol/L *f*-methionyl-leucyl-phenylalanine was used as the stimulus.

selective expression of IL-3Rα chain and the common β chain on endothelial cells. Because we could find no receptors on umbilical vein endothelial cells for GM-CSF or IL-5, the possibility that IL-3 has an important role in endothelial cell biology existed. Indeed IL-3 had a range of effects on endothelium. Some were costimulatory effects with tumor necrosis factor (TNF) or IL-1 (e.g., IL-8 and E-selectin expression), but Khew-Goodall et al. showed that IL-3 by itself had the unique effect of stimulating chronic expression of P-selectin, an adhesion molecule for leukocytes, on endothelial cells.⁷

The importance of the discovery of IL-3 having effects on endothelium relates to the cellular source of IL-3. Thus activated T lymphocytes not

only elaborate general activators of endothelial function such as lymphotoxin, but also IL-3 that has specific effects that endow endothelial cells with unique properties.

Role of IL-3 in eosinophil function

It has been known for some time that IL-3 can powerfully prime the function of eosinophils. Thus, during the cellular interaction of eosinophils and endothelium, the cytokine IL-3 may have an effect on *both* cell types.

To investigate the role of IL-3 in this interaction, Smith and Bernard set up an assay in which the transmigration of eosinophils could be measured in a transwell apparatus. It had been previously demonstrated that endothelial cells acti-

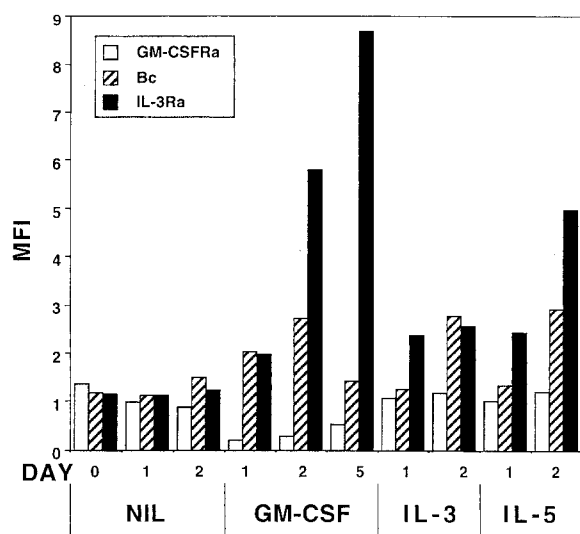


FIG. 1. Expression of receptors for GM-CSF and IL-3 by eosinophils. Eosinophils were stained for flow cytometry analysis with antibodies to GM-CSF-R α or IL-3R α or to the common β chain (β c), either fresh or after culture in medium alone or GM-CSF, IL-3, or IL-5 for the indicated period. MFI, Mean fluorescence intensity.

vated by TNF stimulate the transmigration of neutrophils without the addition of an exogenous chemotactic gradient.⁴ For eosinophils the story began to unfold with the demonstration that resting eosinophils did not readily move in a chemotactic gradient but ones treated (or primed) by treatment for 24 hours with GM-CSF did so readily. However, even those primed cells did not transmigrate through TNF-activated endothelium, but treatment of endothelium with IL-4 caused a strong transmigration of primed but not of unprimed eosinophils (Table IV). By contrast, IL-4 has been shown to inhibit the effects of TNF on endothelium as measured by neutrophil transmigration.⁸

IL-3 treatment of endothelial cells had an interesting effect in the transmigration assay (Table IV). In agreement with previous reports,^{4, 5} TNF did not stimulate the movement of primed or unprimed eosinophils, and IL-4 stimulated the movement of primed but not of unprimed cells. IL-3 by itself had no detectable effect. However, the addition of IL-3 to IL-4 resulted in a novel state of endothelial activation that resulted in the transmigration of unprimed eosinophils.

It thus appears that the repertoire of cytokines elaborated by T lymphocytes is indeed critical in the regulation of allergic inflammation. The Th2 cell type secretes IL-5 (stimulates eosinophil production and primes these cells), IL-4 (activates

endothelium for eosinophil but *inhibits* neutrophil transmigration), and IL-3, which now appears critical to intensify and to prolong inflammation, and to allow the movement of unprimed eosinophils into the tissues.

Therapeutic approaches

Given this panoply of cytokine effects, it appears clear that antagonists would be useful therapeutic agents. With this in mind, Hercus et al.⁹ set about to develop antagonist molecules.

Antibody to IL-3R α chain. An MAb (7G3) was developed against the IL-3R α chain that bound to the α chain with an affinity 10- to 20-times higher than the actual ligand and blocked the function of IL-3. The results in Table V show the antibody inhibiting the priming of basophils by IL-3 for histamine release.

GM-CSF antagonist. A derivative of GM-CSF, with substitution of arginine for glutamic acid in position 21 was made that bound normally to the α chain of the receptor but that failed to allow high-affinity binding by disrupting the association of the ligand-receptor complex with the β chain. This molecule was a specific antagonist of GM-CSF and prevented its function in a range of biologic assays with a potency that could be predicted from its binding to the α chain (Table VI).

This molecule and similar ones for IL-3 and IL-5 have obvious potential in the treatment of allergic disease.

DISCUSSION

The advances in cytokine research have highlighted several areas of importance to allergy. The first relates to the expression of receptors for IL-3 on endothelium and how this molecule may have specific roles in allowing the traffic of eosinophils into tissues. The effect of IL-3 on P-selectin expression already indicated that it had a unique effect on inflammation, potentially explaining aspects of chronicity. Because IL-3 is made by T cells, the control of tissue eosinophils and of blood eosinophils may be primarily T-cell mediated, and IL-3 seems to combine several properties that may make it important in regulation of allergic disease.

Second, the emerging area of the expression of receptors for hemopoietic growth factors has been made possible by the availability of specific MAb. Even preliminary results (Fig. 1) show that these receptors can be rapidly modulated, and their expression may reflect particular activation states of eosinophils or basophils. A speculative

area is the possibility that groups of cytokines stimulate specific patterns of expression of receptors and that this pattern is responsible for behavior of the cell and can be diagnosed by MAb.

Third, the possibility of having clinically relevant antagonists is real with the development of powerful blocking MAb and, perhaps more important, of analogues of growth factors that act as antagonists by specifically binding one of the chains of the receptors for these factors (Tables V and VI). Although each of these reagents may have significant drawbacks in clinical use (the MAb is murine and the antagonist must be used in excess over ligand of approximately 100 times), each points the way for the specific design of smaller molecules that may be more potent and more easily administered.

Finally, the effect of cyclosporine on cells other than T lymphocytes also points the way for the inhibition of transcription of cytokine genes in non-T cells. The particularly attractive possibility is that there exist several pathways of activation only some of the activation pathways are sensitive to cyclosporine. If this were to be the case, it may be possible to target activation pathways specific for allergy.

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The clinical relevance of basophil releasability

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In contrast to many words used in allergy (e.g., *atopy*, *asthma*, *apoptosis*) that derive from ancient Greek, the term *releasability*, recently introduced in the English-language literature, does not exist in most dictionaries. It was first

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Abbreviations used

BAL:	Bronchoalveolar lavage
Con A:	Concanavalin A
D ₂ O:	Deuterium oxide
Der p I:	<i>Dermatophagoides pteronyssinus</i> p I antigen
FMLP:	N-Formyl-methionyl-leucyl-phenyl-alanine
IL:	Interleukin
LTC ₄ :	Leukotriene C ₄
PMC _E :	Lung parenchymae mast cells isolated enzymatically
PMC _M :	Lung parenchymae mast cells isolated mechanically
SCF:	Stem cell factor
Staph A:	Protein A from <i>Staphylococcus aureus</i> Cowan I