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Cytokine expression in allergic inflammation: systematic review of *in vivo* challenge studies

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***Background*: Allergic inflammatory responses are driven by cells of the immune system that rely on cytokines to regulate the activity of other immune and structural cells.**
***Objective*: To review published studies to (1) identify cytokines consistently increased after allergen challenge in atopic patients and (2) investigate temporal variation in cytokine expression.**
***Methods*: A PUBMED systematic search was used to extract data from studies involving analysis of cytokine expression in fluids or biopsies following *in vivo* allergen challenge in atopic patients.**
***Results*: Data were extracted from 82 studies. There were no consistent reports of cytokine protein increase in fluids of patients at 0–1 h after challenge. At 4–12 h, the chemokines eotaxin, macrophage inflammatory protein-1α, RANTES (regulated on activation normal T cell expressed and secreted) and interleukin (IL)-8 have all been consistently reported to be up-regulated. At 18–24 h after challenge, the lymphokines IL-4, IL-5 and IL-13, as well as the pro-inflammatory cytokines granulocyte–macrophage colony-stimulating factor, tumour necrosis factor-α and IL-6 are consistently increased when compared with the respective control value. There were no reports of up-regulation in interferon-γ protein and mRNA and in IL-2 mRNA.**
***Conclusion*: The expression of granulocyte–macrophage colony-stimulating factor is consistently increased in tissues at 4–12 h after challenge. The influence of this cytokine on antigen capture and presentation by dendritic cells should be further investigated. Additionally, allergen challenge studies are needed that investigate the expression of macrophage-derived chemokine and thymus-regulated and activation-regulated chemokine in tissues of atopic patients. Blocking the effects of these lymphocyte-specific chemokines might provide new therapeutic approaches for the control of allergic inflammation.**

Key words: Allergen challenge, Cytokine, Inflammation, Allergy

Introduction

Allergic reactions result from an inappropriate response of the immune system towards a group of non-self-molecules collectively termed allergens.[1] This over-reaction of specific defence mechanisms invariably triggers an inflammatory response that in some cases can cause debilitating secondary effects such as asthma.[2] Allergic inflammatory responses involve both cells of the immune system and structural cells of the injured tissue. To efficiently restore homeostasis, the activity of these cells must be integrated and coordinated. To a great extent, this coordination involves cellular communication via cytokine release.

Cytokines are extracellular signalling proteins produced by different cell types that act on target cells to modulate diverse cellular functions.[3] Some cytokines recruit specific cell types to the site of inflammation.[4] Others can act on immune cells to increase activation and survival,[5,6] whereas some, like interferon (IFN)-γ, suppress cellular activity.[7] The role of cytokines in mounting an adequate immune response is further supported by the ability of lymphokines like interleukin (IL)-4 and IL-13 to induce immunoglobulin isotype switch in B cells[8,9] and to modulate T-cell differentiation.[10] Therefore, identifying the pattern of cytokine expression observed during the development of an allergic inflammatory response provides a valuable insight to the underlying immunological mechanisms.

Bronchial, nasal and cutaneous allergen challenge tests have been extensively used as a means of investigating allergen-induced inflammation.[11] Since the first studies published in 1991,[12,13] literally hundreds of trials have investigated cytokine profile

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following *in vivo* allergen challenge. In some cases, conflicting results have been reported. This review summarises data from these studies addressing the following questions: Which cytokines are consistently up-regulated in allergen challenged tissues of atopic patients? Is there a time-dependent pattern of cytokine expression? Finally, we discuss how these results can be used to improve the understanding and the control of allergic diseases.

Methods

Literature search

The PUBMED database was searched using the query "(allergen) AND (biopsy OR biopsies OR lavage* OR fluid* OR section*) AND (interleukin* OR IL- OR cytokine* OR chemokine* OR lymphokine*)", using the limits Text Word, English and Human. This query was performed in May 2002 and returned 292 titles and/or abstracts.

Literature selection

The abstracts of these 292 articles were screened for provisional inclusion in this review. Articles were dropped from further analysis if they clearly did not involve: (1) human subjects, (2) allergen challenge *in vivo* and (3) collection of bronchial, nasal or cutaneous biopsies and/or fluids; review articles were also eliminated at this step. This procedure yielded 147 articles,[12–158] of which five could not be obtained in full text format.[154–158] The full text of the remaining 142 articles was screened to identify studies that included clinical trials involving: (i) the criteria (1)–(3) described earlier, (ii) a cytokine baseline or control value for each subject, and (iii) appropriate cytokine quantitative data. This procedure resulted in 107 relevant studies[12–118] that were used for data extraction. The main criterion for exclusion in this last step was the absence of the aforementioned (iii) (51%). Inappropriate data commonly resulted from the absence of statistical comparison between baseline or control cytokine values and the post-allergen challenge value.

Data extraction

Several variables were extracted to characterise the subjects and the clinical trial used in each of the 107 selected studies. These included number, disease and atopy status of subjects challenged with allergen and, to characterise the trial design, the challenge site (lung/nose/skin), the allergen used, the specimen collected (biopsy/fluid), the control used (baseline/saline) and the time point of specimen collection after the allergen challenge (0–1 h/4–12 h/18–24 h). In addition to these descriptive variables, the following information for each cytokine analysed was also extracted: control and post-allergen cytokine quantification, method for quantification and significance of the variation (p value).

Data analysis

Analysis was restricted to patients; data from healthy control subjects were not sufficient to allow proper analysis. Data from patients undertaking immunotherapy or corticosteroid treatment were also discarded. Each study contributed data more than once to the analysis if it involved: (a) two or more independent subject samples, (b) both biopsy and fluid collection per subject sample, (c) two or more cytokines analysed per specimen, or (d) for a given cytokine, a specimen collected at two or more time points after allergen challenge. When more than one specimen was collected and analysed for the same time point (e.g. report of cytokine level at 6 and 8 h after allergen challenge), the largest of the values was selected. This approach resulted in 358 contributions extracted from 82 published articles.

Of these 358 contributions, 205 reported a significant positive variation of the cytokine level when comparing the post-allergen value with the control value. By contrast, 149 articles reported no significant variation and four reported a significant negative variation. Therefore, the two latter categories were collapsed into one category (no increase) for statistical analysis.

This review does not involve a meta-regression analysis. Rather, it provides a convenient summary of studies that addressed similar questions using similar study designs. To complement such a summary, a binomial test for proportions was calculated to assess the likelihood of obtaining the observed cytokine data (i.e. number of studies reporting a significant positive variation versus number of studies reporting no increase) given an expected value predicted by the null hypothesis (H_0). The H_0 was that the allergen challenge had no effect on the cytokine level when compared with the control. This H_0 was translated into the binomial probabilities of 0.05 (p, probability of success) and 0.95 (q, probability of no success). An observed set of cytokine data was considered to be unlikely to occur under the H_0 if the probability value resulting from the binomial test for proportions was < 0.001.

Results

Data description

Data reported here refer to 358 contributions extracted from 82 articles (mean contribution per

article, four). These 358 contributions were relatively uniform with respect to the atopy status of the patients and the allergen used for challenge. Indeed, 98% involved a sample of atopic subjects and 91% used a common allergen for challenge (grass, ragweed, birch, dust mite or pet extract). In addition to the allergen type, the allergen concentration used for challenge is known to affect the consequent severity of the inflammatory response. However, there was no consistent report of standardised values of allergen concentration, which prevented such analysis. The post-allergen cytokine value was compared with the baseline or saline challenge value in 39% and 61% of the cases, respectively. The studies reviewed here involved on average 10 subjects per trial (range, 4–35 subjects).

The 358 contributions included 200 fluid and 158 biopsy analyses. Ninety-one per cent and 84% of these analysed cytokine protein level by immunoassays (mostly enzyme-linked immunosorbent assay) and cytokine mRNA by *in situ* hybridisation, respectively. The remaining contributions involved different methods (e.g. immunohistochemistry) and, therefore, were not used for subsequent analysis.

Cytokine protein expression in fluids

Studies that assessed free protein levels in fluids provided quantitative data for 20 cytokines. However, for only 13 cytokines was the sample size (i.e. number of contributions) large enough to assure sufficient power to reject the null hypothesis (power ≥ 0.9). Summary data for these cytokines, as well as the references that provided the information, are presented in Table 1.

Four points should be noted. First, there are eight cytokines for which expression was analysed within 0–1 h after allergen challenge. For all these, no consistent positive variation was reported. Second, six cytokines appear to be consistently up-regulated in fluids at 4–12 h after challenge. Four of these are chemokines (eotaxin, macrophage inflammatory protein (MIP)-1α, RANTES (regulated on activation normal T cell expressed and secreted) and IL-8). Third, 18–24 h after challenge, the lymphokines IL-4, IL-5 and IL-13, as well as the pro-inflammatory cytokines granulocyte–macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor (TNF)-α and IL-6 are consistently increased compared with the respective control value. Finally, there was no evidence that IFN-γ is increased after challenge, whereas two studies demonstrated a significant IL-2 increase. Cytokines reported by a small number of studies ($n \leq 2$) are not presented in Table 1; these include monocyte chemotactic protein (MCP)-1,[45,118] MCP-3,[25] macrophage-derived chemokine (MDC) and thymus-regulated and activation-regulated chemokine (TARC),[16] transforming growth factor-α,[65] IL-1α,[39] and IL-16.[58,92]

Cytokine mRNA expression in biopsies

Most studies analysing cytokine mRNA in biopsies quantified the number of mRNA-positive cells per area of allergen-challenged tissue. This was usually conducted 18–24 h after challenge (Table 2). Of special interest is the consistent report of significant increases in mRNA positive cells for GM-CSF, IL-4, IL-5 and IL-3 and, by contrast, the systematic reports of no variation in IL-2 and IFN-γ. The expression of IL-10 and IL-13 mRNA was also shown to be up-regulated by independent studies. Cytokines not presented in Table 2 due to small sample size include eotaxin,[20,69] MCP-3,[111] MIP-1α,[114] RANTES, IL-6 and IL-8,[54,55] IL-12,[109] and IL-16.[60]

Discussion

This systematic review provides a summary of the published data reporting cytokine expression following allergen-induced tissue inflammation. It aimed to address two questions: Which cytokines are consistently up-regulated in fluids or biopsies of allergen challenged tissues in atopic patients? Is there a time-dependent pattern of cytokine expression? Here, we will point out how this knowledge can be used to improve the understanding and the control of allergic diseases.

First, no evidence could be found that cytokines are consistently increased within 0–1 h after allergen challenge. Within this period atopic subjects are known to experience an immediate hypersensitive response, which is driven to a great extent by mast cells and results in considerable tissue damage.[159] Since mast cells are known to release preformed inflammatory cytokines upon activation,[160,161] it is to some extent surprising that no cytokine was observed to be consistently up-regulated within 0–1 h after challenge.

Second, this review confirms previous individual reports that the chemotactic cytokines eotaxin, MIP-1α, RANTES and IL-8 are increased at a time point that corresponds to the onset of the late phase allergic reaction (4–12 h; see reference 11). Third, the inflammatory cytokines GM-CSF, TNF-α and IL-6, and the lymphokines IL-3, IL-4 and IL-5 are found to be significantly increased across a large number of independent studies at 18–24 h. Finally, IFN-γ protein and mRNA, and IL-2 mRNA have not been reported to be increased by any of the studies here reviewed. Although an attempt has been made to avoid labelling cytokines as Th1-like or Th2-like, the combined results presented here agree with the Th2

Table 1. Variation of cytokine protein levels in fluids collected after allergen challenge in atopic patients.

	Positive variation† *n**N*	Disease status‡ asthma\rhinitis\other	Challenge site§ Lung\nose\skin	References
Eotaxin				
4–12 h	4\6*	2\2\2	2\2\2	20,26,34,62,76,92
GM-CSF				
0–1 h	1\7	2\5\0	2\5\0	39,49,63,64,89,101
4–12 h	5\8*	1\5\2	0\5\3	33,34,63,66,76,89
18–24 h	7\9*	9\0\0	8\1\0	12,33,42,49,65,70,101,108
IFN-γ				
18–24 h	0\3	3\0\0	3\0\0	65,101,102
MIP-1α				
4–12 h	3\3*	1\1\1	1\2\0	26,45,89
RANTES				
0–1 h	1\3	0\1\2	0\2\1	76,89,92
4–12 h	5\6*	2\1\3	2\3\1	26,45,89,92,95,118
18–24 h	2\3	2\0\1	2\1\0	26,45,90
TNF-α				
0–1 h	1\4	1\3\0	1\3\0	17,39,101
4–12 h	0\3	0\2\1	0\2\1	39,118
18–24 h	6\6*	5\1\0	5\1\0	42,70,88,101,102
IL-1β				
0–1 h	1\3	1\2\0	1\2\0	64,89,101
18–24 h	2\4	4\0\0	4\0\0	42,70,101
IL-2				
18–24 h	2\3	2\1\0	2\01	61,65,101
IL-4				
0–1 h	0\4	3\1\0	3\0\1	35,49,56,101
4–12 h	3\3*	0\3\0	0\1\2	35,76,104
18–24 h	7\10*	8\2\0	8\2\0	42,41,44,56,65,70,88,101,102,104
IL-5				
0–1 h	1\5	2\2\1	3\2\0	49,51,63,92,101
4–12 h	3\6	2\3\1	2\3\1	34,43,63,82,92,93
18–24 h	10\12*	10\2\0	10\2\0	42,49,52,65,70,82,88,90,93,101,102
IL-6				
0–1 h	1\4	1\2\1	1\2\1	39,101,118
4–12 h	3\6	1\4\1	0\4\2	33,39,61,104,118
18–24 h	6\6*	5\1\0	4\2\0	33,42,70,101,104
IL-8				
0–1 h	1\9	2\4\3	3\5\1	38,48,74,89,101,105,118
4–12 h	7\11*	4\4\3	3\7\1	53,38,48,74,80,82,89,96,118
18–24 h	4\8*	7\1\0	5\3\0	33,42,70,80,82,96,101
IL-13				
18–24 h	3\3*	3\0\0	3\0\0	47,56,102

Each study involved both a control and a post-allergen challenge protein assay. The latter was performed in fluids collected 0–1 h, 4–12 h or 18–24 h after challenge. The number of studies (i.e. contributions, since some studies involve more then one subject sample; see Material and methods for details) reporting a significant higher level of protein after challenge when compared with the control value is represented by *n*.
† Number of studies (*n*) reporting a significant positive variation of cytokine level following allergen challenge. N: total number of studies reporting cytokine data.
‡ Number of studies involving patients with asthma, rhinitis or other diseases.
§ Number of studies involving allergen challenge in the lung, nose or skin.
* $p < 0.001$, binomial test for proportions (see Methods and methods for details).

profile claimed by many to be involved in asthma or other allergic diseases.

How can the conclusions from these studies be used to improve the understanding and control of allergic reactions?

Allergen challenge studies and the immunobiology of allergic reactions

Different studies have highlighted the fact that inflammatory cytokines are significantly increased in tissues exposed to allergen. These could be considered the danger messages used by cells of the immune system to sense a break in homeostasis. One of such cytokines identified by allergen challenge studies is GM-CSF. This cytokine is secreted by a variety of cells, including T cells, macrophages and structural cells of the injured tissue.[162,163] It acts pleiotropically with effects on target cells that include proliferation and differentiation of haematopoietic cells and activation of eosinophils.[164,165] Importantly, GM-CSF has recently been shown to stimulate the functional maturation and activation of dendritic cells (DC) *in vivo*.[166,167] Together, these findings might provide an important insight to the understanding of the immunobiology of allergic reactions. Indeed, DC are essential in the control of immunity.[168] Although subject to considerable research at present,[169] the maturation state of DC at the time of antigen presentation to T cells in the lymph nodes seems to be a key step that determines whether the organism will develop tolerance or immunity against self-

Table 2. Variation of cytokine mRNA expression in biopsies collected after allergen challenge in atopic patients.

	Positive variation† *n**N*	Disease status‡ asthma\rhinitis\other	Challenge site§ lung\nose\skin	References
GM-CSF				
18–24 h	7\7*	2\2\3	1\4\2	15,30,32,72,79,91,100
IFN-γ				
0–1 h	0\3	0\2\1	0\2\1	54,55,99
18–24 h	0\10	1\4\5	1\6\3	15,30,32,54,55,68,72,99,100,112
TNF-α				
18–24 h	2\4	0\2\2	0\3\1	54,55,115
IL-2				
0–1 h	0\3	0\2\1	0\2\1	54,55,99
18–24 h	0\8	1\2\5	1\5\2	15,30,32,54,55,68,99,100
IL-3				
18–24 h	5\7*	2\2\3	1\4\2	15,30,32,54,55,91,100
IL-4				
0–1 h	1\3	0\2\1	0\2\1	54,55,99
4–12 h	4\5*	2\1\2	1\1\3	14,43,78,99,110
18–24 h	15\18*	4\5\9	2\9\7	14,15,24–32,37,54,55,68,72,77,91,99,100,110,112,116
Il-5				
0–1 h	1\3	0\2\1	0\2\1	54,55,99
4–12 h	2\4	2\1\1	1\1\2	14,43,78,99
18–24 h	10\13*	3\5\5	1\7\5	14,15,30,32,54,55,68,72,91,99,100,112,117
IL-10				
0–1 h	2\3	1\2\0	0\2\1	54,55,87
IL-13				
18–24 h	2\4	0\3\1	0\4\0	36,54,55,72

Cytokine mRNA expression was assessed by in *situ* hybridization. Each study compared the number of positive cells observed in biopsies taken after challenge with the respective value observed in the control biopsy.
† Number of studies (*n*) reporting a significant positive variation of cytokine level following allergen challenge. N: total number of studies reporting cytokine data.
‡ Number of studies involving patients with asthma, rhinitis or other diseases.
§ Number of studies involving allergen challenge in the lung, nose or skin.
* $p < 0.001$, binomial test for proportions (see Methods and methods for details).

molecules and non-self-molecules.[169,170] DC mature in peripheral tissues that secrete GM-CSF in response to injury. Therefore, it is plausible that the stimulation that DC receive *in situ* from this cytokine is a crucial step that determines the efficiency of these cells to prime antigen-specific immune responses. Importantly, the antigen presentation ability of DC might not only be a function of the GM-CSF concentration present in the microenvironment where it captured antigen, but also a function of the GM-CSF receptor allele that the cell expresses. This hypothesis has recently received some support from patients with lupus erythematosus. Indeed, dysregulation of the GM-CSF receptor in antigen presenting cells was found to be associated with a down-regulation of the CD80 costimulatory antigen.[171] This could in turn lead to a dysfunction of antigen presentation to T cells. Although the GM-CSF receptor has been implicated in lung homeostasis,[172–174] its role in the development of primary immune responses is still largely unknown.

Allergen challenge studies and the control of allergic reactions

Allergen challenge studies have demonstrated that chemotactic cytokines and lymphokines are significantly increased as a result of allergen challenge. Chemokines, such as eotaxin and MIP-1α, are released by immune and structural cells of the injured tissue[175,176] and are responsible for the late recruitment of eosinophils, neutrophils and basophils to the site of inflammation.[177,178] This recruitment is an essential step in the development of the late-phase allergic response and, therefore, it represents a candidate immunological mechanism to be targeted by new therapeutical approaches. On the contrary, MDC and TARC are potent attractants of polarised Th2 cells.[179,180] Although the 'Th2 hypothesis' for asthma or other allergic diseases is likely to be too simplistic,[181,182] efficiently blocking the effects of these cytokines could represent a plausible approach to reduce the up-regulation of lymphokines such as IL-4, IL-5 and IL-13. Therapeutic approaches involving blocking antibodies against specific cytokines characteristic of allergic reactions have recently been reported. Indeed, the injection of a monoclonal antibody to IL-5 in allergic asthmatic volunteers significantly reduced the number of eosinophils in blood and sputum.[183] Although no changes were observed in the magnitude of the late-phase response, this clearly demonstrated that efficient *in vivo* blockage of cytokine effects in allergic asthmatics can drastically influence specific immunological mechanisms.

This review confirms that inflammatory cytokines such as GM-CSF, TNF-α and IL-6 are increased in tissues of atopic patients exposed to allergen. There-

fore, the ability of these cytokines to modulate antigen capture and antigen presentation *in vivo* by DC should be further investigated. In addition, it is also shown that different lymphocyte-derived cytokines are significantly increased after allergen challenge. Thus, the development of new strategies for allergic disease control should consider the effects of blocking chemokines that target specific lymphocyte subtypes, such as MDC or TARC. Data concerning the expression of these chemokines following allergen exposure is scarce and, therefore, should be addressed by future allergen challenge studies.

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