

Letter to the Editor

Increased IFN- γ activity in seasonal allergic rhinitis is decreased by corticosteroid treatment

To the Editor:

The allergic response is orchestrated by CD4⁺ T lymphocytes that release T_H2 cytokines when exposed to allergen peptides. According to the T_H2 paradigm, this response is counterregulated by T_H1 cells through the release of IFN- γ (IFNG).¹ Although increasing evidence shows that other T-cell subsets also play important roles, the clinical relevance of the T_H2 paradigm for allergic disease is supported by a large number of reports.² However, *in vivo* studies have shown variable and even increased IFN- γ levels in patients with allergy.²⁻⁵ In our own studies, IFN- γ increased in some patients with seasonal allergy rhinitis (SAR) during the pollen season.³ This could be explained by factors like mixed cell populations or variable dilution of nasal fluids, which makes the interpretation of local IFN- γ levels difficult. On the other hand, experimental data indicate that T_H1 signaling is required for T_H2-cell differentiation and airway inflammation.^{6,7} Moreover, IFN- γ may activate other genes and cells that are involved in allergic inflammation. This is supported by clinical observations, such as a positive correlations between nasal fluid IFN- γ and eosinophil cationic protein (ECP) levels in patients with SAR as well between IFN- γ and disease severity in asthma.^{3,5} SAR is an optimal model of the human allergic response in that the allergen is known and its effects can be studied *in vivo* in nasal fluid cells and *in vitro* in CD4⁺ cells from patients with SAR. Here we examined whether IFN- γ activity in nasal fluid cells and CD4⁺ cells from patients with SAR would (1) change after activation with allergen and (2) be reversed after treatment with corticosteroids. However, the expression levels of an individual cytokine like IFN- γ give limited information about its activity. Gene expression microarrays (GEMs) have the unique advantage of permitting the analysis not only of IFN- γ but also of its activity *in vivo* and *in vitro* in clinical samples by determining the expression levels of IFN- γ -induced genes. We therefore also used GEM to investigate IFN- γ activity in nasal fluid cells and CD4⁺ cells from patients with SAR.

Patients with birch and/or grass pollen-induced SAR as well as healthy controls were defined according to strict criteria.^{3,4} CD4⁺ cells were obtained from asymptomatic patients outside the pollen season (n = 19). Nasal fluids cells were obtained from symptomatic patients (n = 15) and healthy controls (n = 28) during the season. All antiallergic medication, except the occasional use of cromoglycate eye drops (not used during the last 24 hours), was withheld until the nasal lavage was performed. For the study of CD4⁺ cells, PBMCs from 19 patients were challenged with diluent, allergen (ALK Abelló, Hørsholm, Denmark; 100 ug/mL at a density of 10⁶ cells/mL), or allergen and hydrocortisone (at a 10⁻⁷ mol/L concentration) for 7 days.⁴ CD4⁺ cells were enriched and analyzed with 1 Illumina GEM (Illumina, San Diego, Calif) per patient and condition. IFN- γ , granzyme (GZM)-A, and GZMB in cell supernatants were analyzed with ELISAs (R&D Systems Ltd, Abingdon, United Kingdom). mRNA from all nasal fluid cells was obtained according to a standardized protocol, amplified, and pooled into 1 patient and 1 control pool for analysis with Affymetrix HuGe U133A GEM (Affymetrix, Inc, Santa Clara, Calif).^{3,4} Genes that showed a more than 2-fold difference in expression were considered differentially expressed; a Student

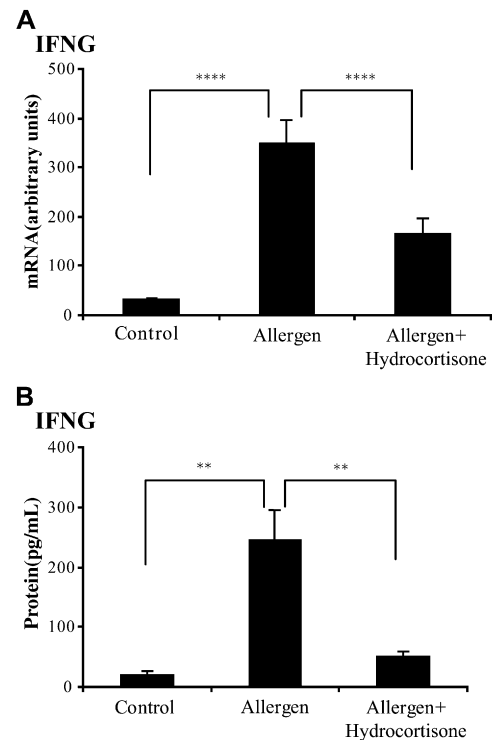


FIG 1. The mean $\pm$ SEM mRNA (A) and protein levels (B) of IFN- γ (IFNG) in allergen-challenged CD4⁺ cells. A, n = 12 patients; B, n = 20 patients. ** P < .01; **** P < .0001.

t test was used to examine whether the fold changes of IFN- γ -regulated genes differed from other genes. The Wilcoxon signed-rank test was used for paired and the Mann-Whitney *U* test for unpaired comparisons. A list of 496 IFN- γ -induced genes was defined by using the Ingenuity pathway analysis program (<http://www.ingenuity.com>; Ingenuity Systems, Inc, Calif). A χ^2 test was used to examine whether more IFN- γ -induced genes than expected by chance were differentially expressed.

Gene expression microarray analysis of CD4⁺ cells showed significant increases of IFN- γ levels after allergen challenge, which decreased after hydrocortisone treatment (P < .0001; Fig 1, A). Of the 24,000 genes whose mRNA expression was analyzed by the GEM, IFN- γ ranked as the 12th most differentially expressed after allergen challenge, and the 77th after hydrocortisone treatment. IFN- γ protein levels also increased in the supernatants of allergen-challenged CD4⁺ cells and decreased after treatment (P < .01; Fig 1, B).

The expression levels of 497 probes for genes known to be induced by IFN- γ were analyzed. A total of 296 changed significantly (Fig 2). This was a significant proportion (P < .0001). Several of the 296 genes had complex inflammatory effects—for example, *TNF*, *IL1A*, *IL1B*, *GZMB*. *GZMB* showed the most significant increase after challenge, as well as reversal after treatment (both P < .0001). A similar pattern was found for *GZMA* (P < .0001). Analysis of *GZMA* and B protein levels in the cell supernatants also conformed to this pattern, both P < .001 (Fig 3, B). A significant proportion of IFN- γ -induced genes were also differentially expressed after hydrocortisone treatment (P < .0001).

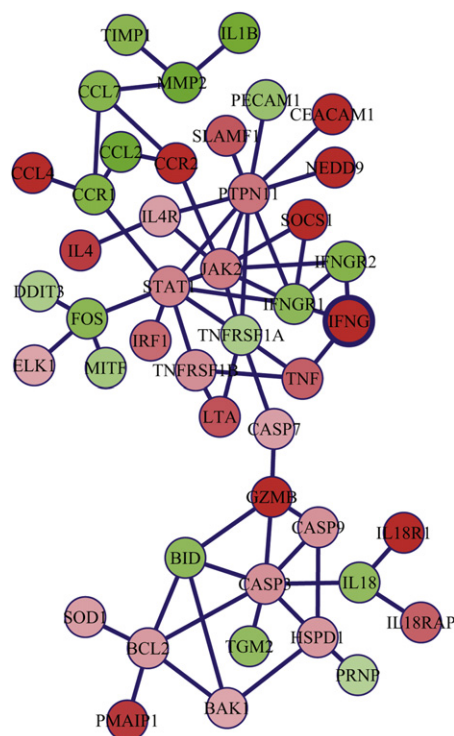


FIG 2. A module of IFN- γ (*IFNG*)-induced genes that were highly differentially expressed in allergen-challenged CD4⁺ cells (adjusted $P < .01$). $n = 12$ patients.

Gene expression microarray analysis of nasal fluid cells from symptomatic patients with SAR during the pollen season also showed increased activity of IFN- γ -induced genes compared with nasal fluid cells from controls ($P < .0001$).

Taken together, these data showed increased IFN- γ activity in SAR. IFN- γ ranked as one of the most differentially expressed genes. By comparison, T_H2-related genes like *GATA3*, *IL4R* and signal transducer and activator of transcription 6 had lower ranks. These findings are not incompatible with a counterregulatory role of IFN- γ in SAR. For example, the IFN- γ -induced genes included *GZMB* which specifically increases apoptosis of T_H2 cells.⁸ Thus, increased IFN- γ activity in SAR could prevent an excessive T_H2 response. However, a more complex role of IFN- γ in SAR is indicated by the large number of IFN- γ -induced genes in both CD4⁺ cells and nasal fluid cells. These genes could influence many cells other than T_H1 and T_H2 cells. Examples include *TNF*, *IL1A*, and *IL1B*, which are pleiotropic inflammatory regulators.

Because corticosteroids have beneficial effects on SAR, hydrocortisone would be expected to repress proinflammatory genes and activate anti-inflammatory genes.⁹ We found decreased IFN- γ activity after treatment, which does not agree with a strictly inhibitory role of IFN- γ in SAR. This was not a result of a general downregulatory effect of corticosteroids, because 57% of the differentially expressed genes after treatment increased in expression.

In summary, increased IFN- γ activity suggests a complex rather than a strictly inhibitory role of IFN- γ in SAR.

Hui Wang, MM^{a*}
 Fredrik Barrenäs MS^{a*}
 Sören Bruhn PhD^a
 Reza Mobini, PhD^a
 Mikael Benson, MD, PhD^{a,b}

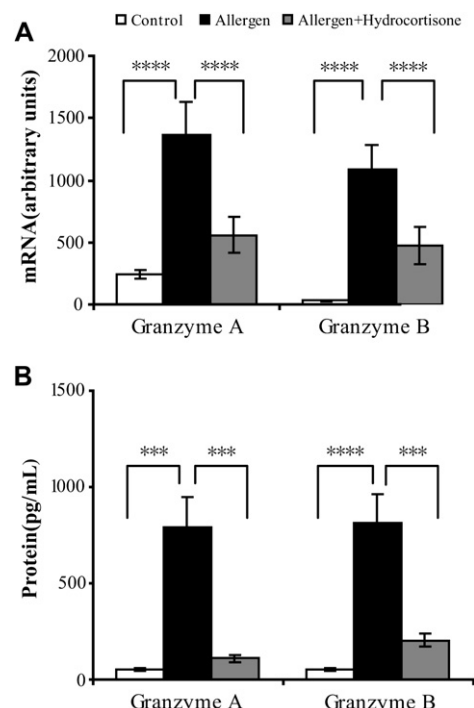


FIG 3. The mRNA (A) and protein levels (B) of GZMA and GZMB in allergen-challenged CD4⁺ cells. A, $n = 12$ patients; B, $n = 20$ patients. *** $P < .001$; **** $P < .0001$.

From ^athe Unit for Clinical Systems Biology, Queen Silvia Children's Hospital, University of Gothenburg, and ^bthe Pediatric Allergy Unit, Queen Silvia Children's Hospital, Gothenburg, Sweden. E-mail: hui.wang@gu.se.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

*These authors contributed equally to this work.

REFERENCES

1. Pernis A, Gupta S, Gollob KJ, Garfein E, Coffman RL, Schindler C, et al. Lack of interferon gamma receptor beta chain and the prevention of interferon gamma signaling in TH1 cells. *Science* 1995;269:245-7.
2. Woodfolk JA. T-cell responses to allergens. *J Allergy Clin Immunol* 2007;119:280-94.
3. Benson M, Strannegård IL, Strannegård O, Wennergren G. Topical steroid treatment of allergic rhinitis decreases nasal fluid TH2 cytokines, eosinophils, eosinophil cationic protein, and IgE but has no significant effect on IFN-gamma, IL-1beta, TNF-alpha, or neutrophils. *J Allergy Clin Immunol* 2000;106:307-12.
4. Benson M, Langston MA, Adner M, Andersson B, Torinsson-Nalwai A, Cardell LO. A network-based analysis of the late-phase reaction of the skin. *J Allergy Clin Immunol* 2006;118:220-5.
5. Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4⁺ and CD8⁺ T cells in atopic asthma. *Am J Respir Crit Care Med* 2005;171:224-30.
6. Bocek P, Fouchas G, Paul WE. Interferon gamma enhances both in vitro and in vivo priming of CD41 T cells for IL-4 production. *J Exp Med* 2004;199:1619-30.
7. Sugimoto T, Ishikawa Y, Yoshimoto T, Hayashi N, Fujimoto J, Nakanishi K. Interleukin 18 acts on memory T helper cells type 1 to induce airway inflammation and hyperresponsiveness in a naive host mouse. *J Exp Med* 2004;199:535-45.
8. Devadas S, Das J, Liu C, Zhang L, Roberts AI, Pan Z, et al. Granzyme B is critical for T cell receptor-induced cell death of type 2 helper T cells. *Immunity* 2006;25:237-47.
9. Benson M, Carlsson L, Adner M, Jernäs M, Rudemo M, Sjögren A, et al. Gene profiling reveals increased expression of uteroglobin and other anti-inflammatory genes in glucocorticoid-treated nasal polyps. *J Allergy Clin Immunol* 2004;113:1137-43.