Interferon-alpha affects the tumour necrosis factor-alpha content of mast cells in human nasal mucosa. A pilot study in allergic patients

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SUMMARY

Human nasal mucosal mast cells contain and secrete tumour necrosis factor (TNF)-alpha, which in turn can stimulate histamine secretion by these cells. Interferon (IFN)-alpha can inhibit TNF-alpha secretion by mast cells in vitro. We have addressed the interrelationships between IFN-alpha and the content in TNF-alpha and number of mast cells in vivo, in the human nasal mucosa. Biopsies were taken from two healthy control patients, two allergic patients and two more allergic patients treated topically with IFN-alpha for two weeks; biopsies from the last two patients were taken both before and after stimulation with the specific allergen. Mast cells were counted upon tagging with rhodaminated avidin and by indirect immunofluorescence for TNF-alpha. Data were subjected to analysis of variance. Mast cell numbers were significantly lower in all allergic patients than in controls (P<0.001). Upon IFN-alpha treatment, TNF-alpha positive mast cells were less than in allergic, untreated patients and the opposite was true for TNF-alpha negative mast cells (p<0.05). Allergen challenge caused selective, significant decrease only in the number of TNF-alpha negative mast cells (p<0.05). The results suggest that upon topical IFN-alpha treatment: (1) mast cells stores of TNF-alpha in the nasal mucosa of allergic patients are decreased; and (2) only TNF-alpha negative cells degranulate in response to allergen challenge. Therefore, one may expect that such a treatment reduces the TNF-alpha burden to the mucosa in these patients.

INTRODUCTION

Tumour necrosis factor (TNF)-alpha is a multifunctional, pro-inflammatory cytokine involved in the regulation of tissue homeostasis and local immune responses. Mast cells (MCs) have been recognized as a source of TNF-alpha (Gordon and Galli, 1990), besides several cell types (Carswell et al., 1975; Cuturi et al., 1987;
Dubravec et al., 1990; Higuchi et al., 1990; Costa, 1993). TNF-alpha has been found in the MCs of several animal species by pharmacological methods (Young et al., 1987; Richards et al., 1988, Gordon and Galli, 1990). By immunohistochemistry, this cytokine has been localized to the peritoneal MCs of rats and mice (Beil et al., 1994) and to those of the dermis, oral and nasal mucosa of healthy humans (Walsh et al., 1991; Bradding et al., 1995). In the nasal mucosa, approximately 70% of total MCs express immunoreactivity for this molecule (Bacci et al., 1998). Besides being stored in granules, TNF-alpha can be synthesized and constitutively secreted upon immune stimulation (Old, 1988; Richards et al., 1988; Gordon and Galli, 1990). In turn, TNF-alpha stimulates the secretion of histamine by human skin MCs, in vitro (Van Oeverveld et al., 1991); therefore this cytokine can become part of a positive feed-back loop during antigen stimulated MC secretion.

Interferons (IFNs) are cytokines which are efficient in the treatment of infectious diseases and differ for cells of origin, inducing stimuli, chemical structure and effects. Four IFN types are known, i.e. alpha, beta, gamma and omega. All IFNs have antiviral, antiproliferative and immunomodulatory properties and are secreted in response to viral infections and other stimuli. Exposure of cells such as leukocytes and fibroblasts to viruses and double-stranded RNA induces the production of IFN-alpha and omega. The ratios between these IFNs vary with the tissue of origin and animal species. T-lymphocytes produce IFN-gamma in response to antigens, to mitogens such as staphylococcal enterotoxin A or to a combination of phytohaemagglutinin and phorbol esters (Petska, 1994; Adolf, 1995). IFNs cause an increase in the expression of major histocompatibility complex antigens by several cell types, in the activity of natural killer and cytotoxic T cells, and in the secretion of several cytokines including IFNs themselves (Arnaud, 2002).

In the cytokine network, IFN produced by T helper (Th1) cells downregulates Th2 function and IgE production, thus interfering with IgE mediated MC stimulation (Mosmann and Coffman, 1989).

In vitro, IFN-alpha inhibits growth factor-dependent growth of MC progenitor cells (Schenrtharer et al., 2000) and integrin expression by immature human MCs (Schoeler et al., 2003). In the rat this cytokine and other IFN types inhibit antigen stimulated secretion of histamine by connective tissue MCs (Swieter et al., 1989), the proliferation of mature mucosal MCs (Dalicik, 2002), and the unstimulated secretion of TNF-alpha by all MC types (Bissonette et al., 1995). IFN-alpha applied alone stimulates rat peritoneal MCs to mild and slow (within 24 h) degranulation without significant histamine release (Crivellato, 2002). IFN-alpha also inhibits the synthesis of mRNA for TNF-alpha (Enciso et al., 1996).

In vivo, IFN-alpha is beneficial in the treatment of aggressive systemic mastocytosis (Valent et al., 2003; Hauswirth et al., 2004) and hypertrophic scars (Tredget et al., 1998).

Since MCs play a crucial role in IgE mediated inflammation, as occurs in allergic diseases (Wasserman, 1985), and in view of the interrelationships shown in vitro
between IFN-alpha and MC secretion of histamine and TNF-alpha (Bissonette et al., 1995), we have addressed the issue of MC numbers and TNF-alpha content in the nasal mucosa of patients with allergic rhinitis and of the effect of short-time, local administration of IFN-alpha on those items.

MATERIAL AND METHODS

Subjects

The number of subjects under study had to be restricted on the basis of ethical issues. All subjects had to undergo septorhinoplasty under general anaesthesia and gave informed consent to the study. The protocol was carried out following the Italian law and the ethical guidelines of the Italian National Medical Council in force, and with the approval of the Institution ethical committee.

Six patients (all males) entered the study. Allergic patients were diagnosed on the basis of season-rhythmic symptoms, intense cutaneous response to allergen challenge and presence of circulating IgE specific to gramineae. No patient had ever been subjected to specific, desensitizing immunotherapy and no one had assumed anti-histamine drugs, corticosteroids, local or systemic antiinflammatory drugs nor any other medication within two weeks before being recruited into the study.

Two patients (aged 30 and 60 years) were considered as controls; two patients (aged 24 and 35 years) were diagnosed as severe allergic and were not treated with either IFN-alpha or antigens, two patients (aged 19 and 26 years), also diagnosed as severe allergic, were treated with natural IFN-alpha (Alpha Wassermann, Milan, Italy), 4×10^6 U/die for 15 days, and then challenged at surgery, after completing IFN-alpha treatment, with a spray of Gramineae mix (Lofarma, Milan, Italy) to which they were allergic. From each of the last two patients, one biopsy was obtained immediately before antigen challenge and another one immediately after that challenge.

Light microscopy and immunohistochemistry

Biopsies of nasal mucosa were taken from the edge of the inferior turbinate during surgery for septorhinoplasty under general anaesthesia. The specimens were fixed in Mota's fluid, dehydrated in ethanol, cleared in xylene and embedded in paraplast. For bright field microscopy, sections were stained with 0.5% toluidine blue (Gurr, Pool, UK) in distilled water.

For fluorescence microscopy, sections were treated with tetramethylrhodamine isothiocyanate (TRITC)-labelled avidin (Immunotech, Marseille, France), diluted 1:400, which is a specific and sensitive tag for the granules of all MC (Tharp et al., 1985), followed by a polyclonal antibody against human TNF-alpha (Sigma, Milan Italy), diluted 1:50, which was revealed with a fluorescein isothiocyanate (FITC)-
labelled goat anti-rabbit polyclonal antibody (Sigma), diluted 1:32. The sections were examined with a Zeiss Axioskop (Heidelberg, FRG) light microscope equipped for epifluorescence. The specificity of the immune staining was tested by omitting the first antibody or substituting it with irrelevant ones.

**Morphometry and statistics**

Two to seven sections per biopsy, at least 100 μm apart from each other, were used. Mast cells were counted in at least five microscopic fields per biopsy at magnification x40 (field area 0.08 mm²); TNF-alpha positive MCs were counted separately from those positive only for avidin. For statistical analyses, each microscopic field was taken as a sample unit. Each biopsy from the same experimental condition contributed an almost equal number (± 1) of microscopic fields for these analyses. The data were subjected to analysis of variance (ANOVA). The numbers of TNF-alpha positive versus TNF-alpha negative MCs were also analysed by non-parametrical chi square test. All tests were applied two-tailed. For multiple comparisons, i.e. among the several groups of patients, the significance limit was set at $P = 0.001$; for comparisons between couples of values, i.e. TNF-alpha positive versus TNF-alpha negative cells and one experimental group against another, the significance limit was set at $p = 0.05$. Mean values and standard deviation are given as results.

**RESULTS**

By light and fluorescence microscopy, many MC were recognized in the lamina propria of normal human nasal mucosa. These cells were rich in specific granules which stained metachromatically with toluidine blue and were labelled by TRITC-avidin (Fig. 1a). Mast cells were significantly less in allergic patients than in controls (Figs. 1b, 2). Furthermore their numbers in allergic patients treated with IFN-alpha decreased significantly ($p<0.05$) upon allergen challenge (Fig. 2). A vast majority of the MC stained for TNF-alpha in controls (Fig. 1c). The numbers of MC stained for TNF-alpha was significantly lower in allergic patients (Figs. 1d, 3) than in controls ($P<0.001$ by both ANOVA and chi-square test), while the opposite was true for TNF-alpha negative MC (Fig. 3). In allergic patients treated with IFN-alpha the numbers of TNF-alpha positive MC were significantly less ($p<0.05$) than in allergic patients not treated with the cytokine and did not vary significantly upon antigen challenge; on the contrary the numbers of TNF-alpha negative MC in patients treated with IFN-alpha were not significantly different from those in allergic patients not treated with the cytokine and decreased significantly upon antigen challenge ($P<0.05$; Fig. 3).
Fig. 1 — Demonstration of mast cells by rhodaminated avidin in the nasal mucosa of control (a) and allergic subjects (b). Part of these cells were also labelled for TNF-alpha by immunofluorescence with a fluoresceinated probe. (c: control subject; d: allergic subject). Fluorescence microscopy, x 400.

Fig. 2 — Numbers of mast cells per microscopic field of nasal mucosa. The differences among groups of biopsies were significant (p<0.001; ANOVA). Mean and standard deviation among microscopic fields are shown; the columns depict the following: A: controls (N = 25); B: allergic untreated subjects (N = 15); C: allergic subjects treated with IFN-alpha (N = 11); D: allergic subjects treated with IFN-alpha and stimulated with allergen (N = 12).

Fig. 3 — Numbers of mast cells labelled for TNF-alpha (black columns) and unlabelled for this cytokine (white columns) per microscopic field of nasal mucosa. Indications of columns and numbers of fields are as in Figure 2. The differences among groups of biopsies were significant for either type of mast cells (p<0.001; ANOVA and chi-square test).
DISCUSSION

In this research we found a significant reduction of MC numbers in the nasal mucosa of allergic subjects as compared with controls apparently exempt from inflammatory pathology of that mucosa. This reduction depended only on the numbers of TNF-alpha positive MCs. Furthermore, IFN-alpha treatment was followed by a significant decrease in TNF-alpha positive MCs. Upon antigen challenge, in allergic patients pre-treated with this cytokine there was a significant decrease only in the number of TNF-alpha negative MCs. The results for subjects not allergic nor treated pharmacologically (except for anaesthesia) confirm previous reports that TNF-alpha is synthesized and stored in granules by a majority of the MCs of normal human nasal mucosa, in the absence of any stimulation (Gordon and Galli, 1990; Bradding et al., 1995; Bacci et al., 1998). At the light of the known role of MCs in allergic diseases, which is that of secreting factors stimulating local inflammatory responses and consequently disease (Möller et al., 1994), we interpret the observed decrease in MC numbers in allergic patients as the expression of a steady state in which widespread, continuous degranulation is not balanced by regranulation or differentiation of new MCs (Gurish and Boyce, 2002).

Treatment of allergic patients with topical IFN-alpha seemed to shift the nasal mucosal MCs towards cells without histochemically detectable levels of TNF-alpha. These latter cells retained sensitivity to allergen, as indicated by their decrease upon allergen challenge which should be interpreted as degranulation upon secretion, given its quick occurrence. However, in this condition they could secrete no or only minor amounts of TNF-alpha and the positive feedback exerted by this cytokine on histamine secretion would have been weak or none. These results, although limited by the small number of cases imposed by ethical restrictions, suggest that IFN-alpha interferes with the regulation of TNF alpha synthesis by MCs (Bissonnette et al., 1995), leading to an increase in the number of TNF-alpha negative cells, and with allergen-stimulated granule secretion by the TNF-alpha positive cells still present. These results are in line with those showing inhibition by this cytokine of the synthesis of TNF-alpha mRNA by isolated MCs (Enciso et al., 1996). The apparent stability of TNF-alpha positive MCs upon allergen challenge should be appreciated at the light of the results of Bradding et al. (1995), who found that such a challenge stimulated TNF-alpha secretion by MCs in allergic patients not treated with IFN (treatment with this cytokine had not been tested in that study). On the contrary, the results of the present study suggest that, upon IFN-alpha treatment, the allergen stimulated secretion of TNF-alpha by nasal mucosal MCs of allergic patients is reduced. We should like to conclude that treatment with IFN-alpha of the nasal mucosa of patients with allergic rhinitis has the potential to reduce the TNF-alpha burden to the mucosa.

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