Allergology International (2000) **49**: 183–188

Original Article

Involvement of vascular endothelial growth factor in nasal obstruction in patients with nasal allergy

Tetsuji Yamashita,[1] Nobuhisa Terada,[2] Nanako Hamano,[2] Hirohisa Kishi,[2] Noriko Kobayashi,[2] Yukiko Kotani,[1] Masakazu Miura[1] and Akiyoshi Konno[2]

[1]R&D Mitsubishi Kagaku Bio-clinical Laboratories Inc., Tokyo and [2]Department of Otorhinolaryngology, Chiba University, School of Medicine, Chiba, Japan

Abstract

It has recently been shown that vascular endothelial growth factor (VEGF) enhances vascular permeability and that mast cells produce VEGF, suggesting the involvement of VEGF in allergic diseases. In the present study we quantitatively analyzed VEGF in the nasal lavage fluid of patients with nasal allergy. We performed nasal antigen challenge with Japanese cedar pollen antigen in 10 healthy adult volunteers and in 10 cedar pollen IgE-positive patients with nasal allergy. In all patients with nasal allergy, VEGF and histamine levels in the nasal lavage fluid reached a peak 30 min after antigen challenge, then returned to prechallenge values 2 h after antigen challenge. In these patients, the histamine level increased three-fold, while the VEGF level increased 10-fold. However, in all healthy adult volunteers, VEGF and histamine levels did not increase. A stronger correlation was noted between the ratio of decreased nasal cavity volume and the ratio of increased VEGF levels ($R = 0.823$; $P < 0.001$) than between the ratio of nasal cavity volume and the ratio of increased histamine levels ($R = 0.660$; $P < 0.01$). These results suggest that VEGF may contribute to the pathogenesis of nasal obstruction in the early phase of nasal allergy as a new factor involved in increasing vascular permeability.

Key words: mast cells, nasal allergy, nasal antigen challenge test, nasal obstruction, vascular endothelial growth factor, vascular permeability.

Correspondence: Nobuhisa Terada, Department of Otorhinolaryngology, Chiba University, School of Medicine, Inohana 1-8-1, Chuo-ku, Chiba 260-8670, Japan.
Email: terada@med.m.chiba-u.ac.jp

Received 5 November 1999. Accepted for publication 6 April 2000.

Introduction

Patients with nasal allergy, a type I allergic disease, present with major symptoms of sneezing, increased nasal secretion and nasal obstruction. Type I allergy is characterized by anaphylactic reactions caused by the release of chemical mediators from mast cells induced by IgE-antigen crosslinkage. Nasal obstruction is the most significant of the three major symptoms because it is often associated with mouth breathing, snoring, xerostomia and sleep apnea syndrome. A decrease in the nasal cavity volume is caused by vasodilatation and extravascular leakage of plasma components. To investigate the pathogenesis of nasal allergy, we evaluated the chemical mediators in nasal lavage fluid after a single antigen challenge.[1,2]

A growth factor with a regulatory role in angiogenesis was found and termed vascular permeability factor (VPF) by Senger *et al*.[3] It has also been referred to as vascular endothelial growth factor (VEGF) by Ferrara *et al*.[4] Based on the difference in splicing, four types of VEGF ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$) have been detected.[5] In an experimental study using guinea pigs, VEGF increased vascular permeability when injected subcutaneously. In the field of clinical medicine, VEGF has been reported to participate in angiogenesis in solid tumors and diabetic eyes.[6,7] Vascular endothelial growth factor is also found in carcinomatous ascites produced by increased vascular permeability[8] and VEGF is released from various cells, such as tumor cells, epithelial cells, fibroblasts and platelets.[9–12] A recent study has shown that VEGF is also produced by mast cells.[13] This finding suggests that VEGF has an important role in type I allergic disease.

In the present study, we quantitatively analyzed VEGF in the nasal lavage fluid of patients with nasal allergy

before and after nasal antigen challenge. The nasal cavity volume was also measured to evaluate the participation of VEGF in the development of nasal obstruction, the symptom closely related to vascular permeability.

METHODS

Subjects

A total of 10 Japanese cedar pollinosis patients (mean age 34 years; range 26–34 years) including eight males and two females, participated in the present study. Inclusion criteria for Japanese cedar pollinosis patients were clinical symptoms of nasal hypersensitivity during the Japanese cedar pollen-scattering season (January) and positive results in the radioallergosorbent test (RAST; score > 2) for Japanese cedar pollen. Patients who tested positive in the RAST for other pollen antigens were included as subjects if they showed no nasal hypersensitivity outside the Japanese cedar pollen-scattering season. However, all patients testing positive for antigens against house dust and mites were excluded from the study. The nasal cavity was required to be free of septal deviations, nasal spurs and nasal polyps. The control subjects consisted of 10 healthy adult volunteers (mean age 32 years; range 24–38), including six males and four females without a past history of allergic disease. The study was performed in midwinter, out of the pollen season, to avoid natural provocation.[1,2]

Study schedule

An antigen challenge was performed using two pollen disks (total four disks; each disk was saturated with 250 mg protein nitrogen, 0.3 mg total nitrogen of Japanese Cedar) that were placed on the inferior turbinate. Nasal lavage was performed 15 min and immediately before antigen challenge, as well as at 30 min and 2, 4, 8 and 10 h after antigen challenge. Before the study, informed consent was obtained from each subject after adequately explaining the objectives and procedures of the present study.

Nasal lavage

With the patient in a sitting position with his/her head hanging down, a nozzle connected by a tube to a syringe was inserted into a nostril to close the opening tightly and the nasal cavity was washed with 10 mL physiological saline warmed at 37°C by reciprocating the piston of the syringe 10 times on each side. Lavage fluid recovered was measured, mixed thoroughly, filtered through a nylon filter net (Nippon Rikagaku Kikai Co., Tokyo, Japan) to remove mucin and then centrifuged for 20 min at 4°C at 400 *g*. Vascular endothelial growth factor contained within the mucin was ignored. The supernatant was stored at −80°C until assayed for mediator levels. Vascular endothelial growth factor in the nasal fluid was measured with ELISA using a human VEGF immunoassay (R&D Systems, Minneapolis, MN, USA; detection limit 15.6 pg/mL). The kit used can detect two of the four VEGF isoforms, namely $VEGF_{121}$ and $VEGF_{165}$. Histamine was also measured by radioimmunoassay (RIA) using a histamine RIA kit (Eiken Chemical, Tokyo, Japan; detection limit 0.11 ng/mL).

Nasal cavity volume measured by acoustic rhinometry

Acoustic rhinometry is a method used for measuring the area and length of the nasal cavity by acoustic reflection for quantitative determination of nasal cavity geometry.[14] An A1 acoustic rhinometer ('GM' Instruments Ltd, London, UK) was used to determine nasal cavity volume. A range of 8.5–10.5 cm^3 from the wave tube of the acoustic rhinometer (1.5–3.5 cm^3 from the tip of the nosepiece) was used to determine the volume of the nasal cavity. After patients had blown their noses, nosepieces were closely attached to both the left and right nostrils and the volume of the nasal cavity was measured and then recorded on a computer. The percentage change in nasal cavity volume (%) was determined by the formula: (post-challenge nasal cavity volume/(post-challenge nasal cavity volume – prechallenge nasal cavity volume)) × 100.

Statistical analysis

Results are expressed as the mean ± SEM. Comparisons within groups were made using the Wilcoxon signed-rank test for paired comparisons. Correlations were determined using Spearman's rank method (rank correlation coefficient). The statistical tests were performed on Macintosh computers (Apple Computers, Cupertino, CA, USA) using Statview software (Abacus Concepts, Berkeley, CA, USA). $P < 0.05$ was considered statistically significant.

RESULTS

In all patients with nasal allergy, VEGF levels in nasal lavage fluid reached maximum values 30 min after

antigen challenge and returned to their prechallenge values 2 h after nasal antigen challenge. As shown in Fig. 1, the VEGF level in the patient group was 65.6 ± 10.0 pg/mL before antigen challenge and increased to 581 ± 85 pg/mL 30 min after antigen challenge. However, in the control group, no significant differences were noted in VEGF levels before and after antigen challenge (58.6 ± 15.6 and 67.9 ± 30.0 pg/mL, respectively). The histamine level in the patient group increased from 1.4 ± 0.6 ng/mL before antigen challenge to 4.0 ± 0.5 ng/mL 30 min after antigen challenge. In the control group, no significant differences were noted between before and 30 min after antigen challenge (0.9 ± 0.1 ng/mL and 1.1 ± 0.3 ng/mL, respectively; Fig. 1). Compared with levels before antigen challenge, the histamine level increased three-fold after antigen challenge in each patient, while the VEGF level increased 10-fold.

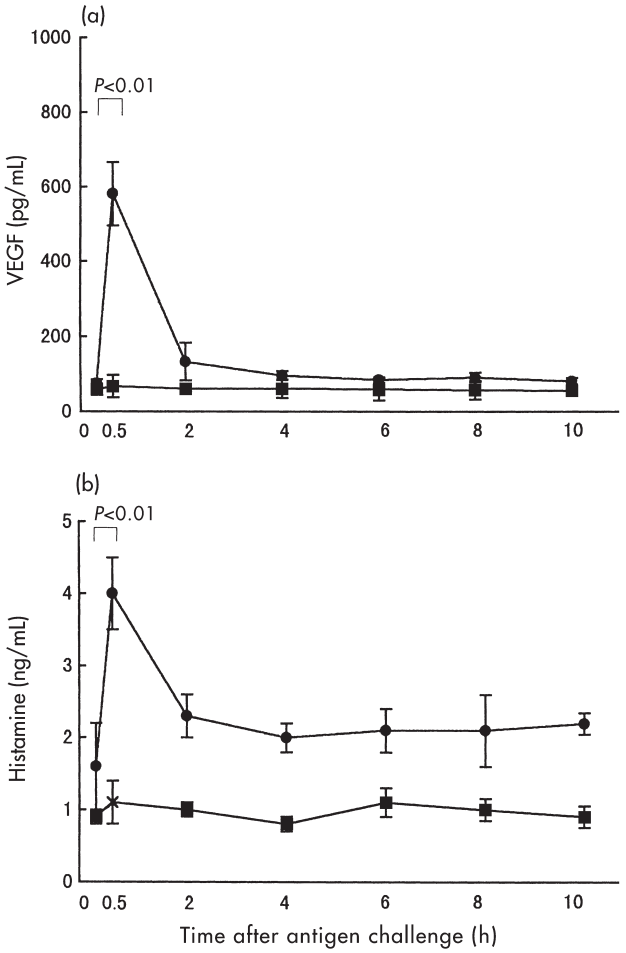

Fig. 1 Mediator levels in nasal lavage fluid after antigen challenge. Antigen challenge was performed using two pollen disks. Nasal lavage was performed 15 min and immediately before antigen challenge, as well as at 30 min and 2, 4, 8 and 10 h after antigen challenge. Data are the mean ± SEM (n = 10). (a) Vascular endothelial growth factor (VEGF). In patients with nasal allergy (●), significant increases occurred immediately after antigen challenge in the first 30 min, with no differences in levels at later hours. However, in the control group (■), no significant difference were observed before and after antigen challenge. (b) Histamine. In patients with nasal allergy (●), significant increases occurred immediately after antigen challenge in the first 30 min, with no significant differences in histamine levels at later time points. However, in the control group (■), no significant differences were observed before and after antigen challenge.

As shown in Fig. 2, the nasal cavity volume before antigen challenge was 3.1 ± 0.3 cm^3, then it reached its minimum value of 2.0 ± 0.3 cm^3 30 min after antigen challenge ($P < 0.01$). In the hours after challenge, a complex pattern of reactions was observed in the allergy group. The pooled data from all allergy patients showed a slight decrease in nasal cavity volume; however, the changes were not statistically significant. In the control group, no significant differences were noted before and after antigen challenge.

The correlation between the ratio of nasal cavity volume decrease and the ratio of VEGF increase ($R = 0.823$; $P < 0.001$) was stronger than that between the ratio of nasal cavity volume and the ratio of increased histamine levels ($R = 0.660$; $P < 0.01$; Fig. 3).

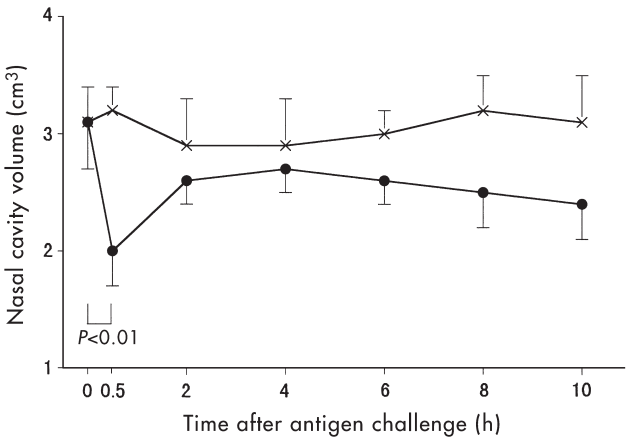

Fig. 2 Changes in nasal cavity volume before and after antigen challenge. A range of 8.5–10.5 cm^3 from the wave tube of the acoustic rhinometer (1.5–3.5 cm^3 from the tip of the nosepiece) was used to determine nasal cavity volume. Data are the mean ± SEM (n = 10). In patients with nasal allergy (●), nasal cavity volume significantly decreased immediately after antigen challenge in the first 30 min. Although some patients showed a decrease in nasal cavity volume at later time points, the pooled data did not reach statistical significance. In the control group (×), no significant changes were observed before and after antigen challenge.

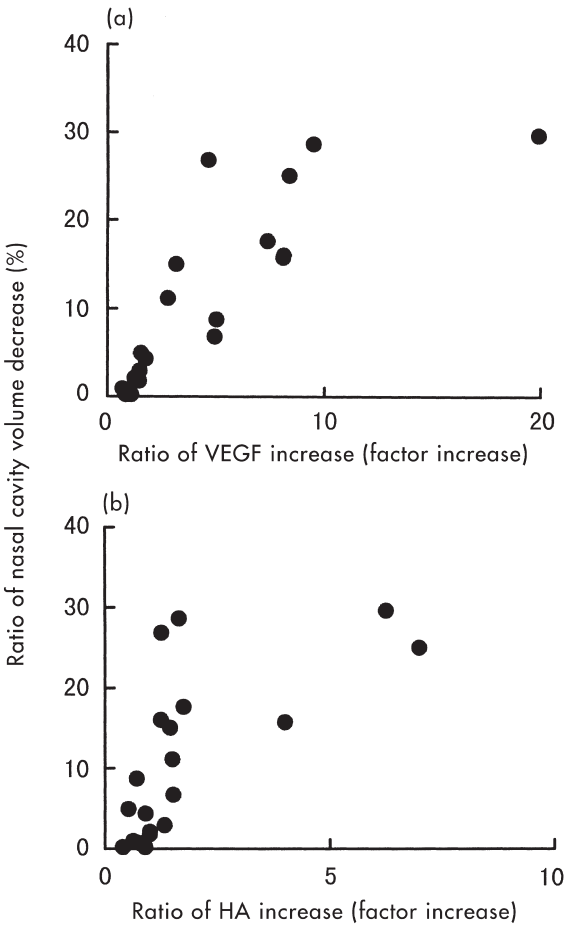

Fig. 3 Correlation between the ratio of nasal cavity volume decrease and the ratio of mediator increase. The correlation between the ratio of nasal cavity volume decrease and (a) the ratio of vascular endothelial growth factor (VEGF) increase was greater than that between the ratio of nasal cavity volume decrease and (b) the ratio of histamine (HA) increase. For (a): $r = 0.823$, $P < 0.001$. For (b): $r = 0.660$, $P < 0.01$.

Discussion

Vascular endothelial growth factor was discovered as a growth factor that specifically acted on vascular endothelial cells.[3,4] It was also found that VEGF was able to enhance vascular permeability. Clinically, it has been reported[6,7,15] that VEGF participates in several conditions caused by abnormal angiogenesis and increased vascular permeability, such as tumor growth and diabetic retinopathy. We have confirmed the presence of VEGF in the fluid of thyroid cysts, in the intra-ocular fluid of patients with diabetic retinopathy and in the peripheral blood of patients with solid tumors.[6,7,15] Recently, knockout mice for VEGF and the VEGF receptor gene were developed to investigate the role of VEGF in angiogenesis during the early developmental stage.[16–18] A neutralizing antibody to VEGF was found to have both antitumor and antimetastatic effects and, thus, is expected to be useful as an anticancer drug.[18,19] Gene therapy based on the physiologic activities of VEGF is now under investigation: namely, transduction of a $VEGF_{165}$ expression vector in patients with arteriosclerosis obliterans and intramuscular injections of VEGF cDNA plasmids in patients with chronic arterial obstruction in the lower extremities.[20–22]

It has recently been reported that VEGF is expressed in keratinosytes during delayed type allergic reactions.[23] However, VEGF has not been reported to directly participate in the development of allergy. Boesiger *et al*.[13] suggested the possibility of VEGF participating in the pathogenesis of allergic disease because VEGF was produced by mast cells in an *in vitro* experiment.

To confirm the presence of VEGF in the nasal cavity with allergic reactions, we measured VEGF and histamine levels in nasal lavage fluid after a single antigen challenge test in patients with nasal allergy. Changes in nasal cavity volume were also measured to examine the correlation between nasal obstruction with VEGF and/or histamine levels. The present study, where the time-course of changes in chemical mediators can be detected, is considered to be suitable for investigating the pathogenesis of nasal allergy.

On the basis of results of the present study, the VEGF level in nasal lavage fluid increased after nasal antigen challenge exclusively in the patient group. The levels of VEGF 30min after antigen challenge increased approximately 10-fold compared with levels before antigen challenge. The levels of VEGF in nasal fluid 30 min after antigen challenge were almost at the same level as in the intra-ocular fluid in diabetic eyes with increased vascular permeability,[7] although a direct comparison between different organs is difficult to make.

Nasal obstruction is, in part, caused by a decrease in nasal cavity volume secondary to interstitial edema due to extravascular leakage of plasma components. Mucosal edema in the nasal cavity is refractory to antihistaminic drugs (first generation histamine H_1 receptor antagonists) in most cases, although it is responsive to topical steroids.[24,25] This is explained by the decrease in the number of mast cells in the nasal mucosa. Unknown factors, other than histamine, released from mast cells may play a role in the development of nasal obstruction because antihistaminic drugs are ineffective in treating

the symptoms, although it has been reported that mucus edema was reduced after medication with mediator release inhibitors in patients with nasal allergy.[26] In addition, transient edema is a side effect of gene therapy with VEGF.[21,22] Therefore, VEGF may cause edema in the nasal mucosa.

In the present study, the histamine level was approximately seven-fold greater than the VEGF level at the peak time after antigen challenge in patients with nasal allergy. However, it has also been reported that the role of VEGF in the enhancement of vascular permeability is 50 000-fold stronger than that of histamine *in vitro*.[27] With regard to inducing increased vascular permeability, VEGF may be more active than histamine, although the metabolism and the density and distribution of the receptors should be considered. Moreover, the correlation between the ratio of decreased nasal cavity volume and the ratio of increased VEGF levels was greater than that between the ratio of decreased nasal cavity volume and the ratio of increased histamine levels.

These results suggest that VEGF is a new factor in the development of nasal obstruction in the early phase. Both VEGF and histamine levels increased in a similar manner and reached their peak values 30 min after antigen challenge. Based on the fact that mast cells produce VEGF, it is certain that VEGF, as well as histamine, in nasal lavage fluid is derived from mast cells in the nasal mucosa. Because the kinetics of VEGF level are similar to those of histamine, there is a possibility that histamine induces VEGF release from mast cells. However, to our knowledge, there is no report regarding the effect of histamine on VEGF production or on mast cell degranulation. In fact, in a preliminary study using cultured mast cells, we were not able to observe an increase in VEGF levels from mast cells when they were cultured with histamine (T Yamashita *et al.*, unpubl. obs., 1999).

Various mediators, including arachidonic acid metabolites (such as platelet-activating factor and leukotrienes) and eosinophil granule proteins, have already been reported to participate in the pathogenesis of late phase response.[1,28] Considering the fact that neither histamine nor VEGF levels were increased in the late phase, they are unlikely to contribute to the nasal swelling in the late phase response.

Consequently, our present study revealed the presence of VEGF in nasal lavage fluid. The results also suggest that VEGF, also known as a vascular permeability factor, possesses a close relationship with the development of nasal obstruction. In the future, VEGF may be one of the targets of therapy for nasal obstruction in patients with nasal allergy.

Acknowledgments

We thank Dr Kinoshita and Dr Koike (Department of Pediatrics, Shinshu University School of Medicine) for their technical support of cultured mast cells.

References

1 Terada N, Konno A, Togawa K. Biochemical properties of eosinophils and their preferential accumulation mechanism in nasal allergy. *J. Allergy Clin. Immunol.* 1994; **94**: 629–42.
2 Terada N, Yamakoshi T, Hasegawa M *et al*. The effect of ramatroban (BAY u 3405), a thromboxane A_2 receptor antagonist, on nasal cavity volume minimum cross-sectional area and nasal mucosal hemodynamics after nasal mucosal allergen challenge in patients with perennial allergic rhinitis. *Acta Otolaryngol. Suppl.* 1998; **537**: 32–7.
3 Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science* 1983; **219**: 983–5.
4 Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 1989; **161**: 851–8.
5 Tischer E, Mitchell R, Hartman T *et al*. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J. Biol. Chem.* 1991; **266**: 11 947–54.
6 Sato K, Miyakawa M, Onoda N *et al*. Increased concentration of vascular endothelial growth factor/vascular permeability factor in cyst fluid of enlarging and recurrent thyroid nodules. *J. Clin. Endocrinol. Metab.* 1997; **82**: 1968–72.
7 Tanaka Y, Katoh S, Hori S, Miura M, Yamashita H. Vascular endothelial growth factor in diabetic retinopathy. *Lancet* 1997; **349**: 1520.
8 Yeo KT, Wang HH, Nagy JA *et al*. Vascular permeability factor (vascular endothelial growth factor) in guinea pig and human tumor and inflammatory effusions. *Cancer Res.* 1993; **53**: 2912–18.
9 Keck PJ, Hauser SD, Krivi G *et al*. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science* 1989; **246**: 1309–12.
10 Clauss M, Gerlach M, Gerlach H *et al*. Vascular permeability factor: A tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. *J Exp. Med.* 1990; **172**: 1535–45.
11 Senger DR, Van de Water L, Brown LF *et al*. Vascular permeability factor (VPF, VEGF) in tumor biology. *Cancer Metast. Rev.* 1993; **12**: 303–24.

12 Mohle R, Green D, Moore MAS, Nachman R, Rafii S. Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc. Natl Acad. Sci. USA* 1997; **94**: 663–8.
13 Boesiger J, Tsai M, Maurer M *et al*. Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of fc epsilon receptor I expression. *J. Exp. Med*. 1998; **188**: 1135–45.
14 Hilberg O, Jackson AC, Swift DL, Pedersen OF. Acoustic rhinometry evaluation of nasal cavity geometry by acoustic reflection. *J. Appl. Physiol*. 1989; **66**: 295–303.
15 Hyodo I, Doi T, Endo H *et al*. Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. *Eur. J. Cancer* 1998; **34**: 2041–5.
16 Ferrara N, Carver-Moore K, Chen H *et al*. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996; **380**: 439–42.
17 Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* 1995; **376**: 66–70.
18 Shalaby F, Rossant J, Yamaguchi TP *et al*. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 1995; **376**: 62–6.
19 Asano M, Yukita A, Matsumoto T, Kondo S, Suzuki H. Inhibition of tumor growth and metastasis by immunoneutralizing monoclonal antibody to human vascular endothelial growth factor/vascular permeability factor121. *Cancer Res*. 1995; **55**: 5296–301.
20 Isner JM, Walsh K, Symes J *et al*. Arterial gene therapy for therapeutic angiogenesis in patients with peripheral artery disease. *Circulation* 1995; **91**: 2687–92.
21 Isner JM, Pieczek A, Schainfeld R *et al*. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. *Lancet* 1996; **348**: 370–4.
22 Baumgartner I, Pieczek A, Manor O. Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation* 1998; **97**: 1114–23.
23 Palacio S, Schmitt D, Viac J. Contact allergens and sodium lauryl sulphate upregulate vascular endothelial growth factor in normal keratinocytes. *Br. J. Dermatol*. 1997; **137**: 540–4.
24 Okuda M, Sakaguchi K, Otsuka H. Intranasal beclomethasone: Mode of action in nasal allergy. *Ann. Allergy* 1983; **50**: 116–20.
25 Otsuka H, Denburg JA, Befus AD *et al*. Effect of beclomethasone dipropionate on nasal metachromatic cell sub-populations. *Clin. Allergy* 1986; **16**: 589–95.
26 Tallat MA, Inaam PK, Mohamed MH *et al*. The histological and histochemical effects of ketotifen in allergic rhinitis. *J. Asthma* 1991; **28**: 117–28.
27 Shulman K, Rosen S, Tognazzi K, Manseau EJ, Brown LF. Expression of vascular permeability factor (VPF/VEGF) is altered in many glomerular diseases. *J. Am. Soc. Nephrol*. 1996; **7**: 661–6.
28 Otsuka H, Inaba M, Fujikura T, Kunimoto M. Histochemical and functional characteristics of metachromatic cells in the nasal epithelium in allergic rhinitis: Studies of nasal scrapings and their dispersed cells. *J. Allergy Clin. Immunol*. 1995; **4**: 528–36.