Hyperresponsiveness of Granulocytes to Anaphylatoxins, C5a and C3a, in Churg-Strauss Syndrome

Takuo Tanaka, Masayoshi Abe, Takashi Mitsuyama, Yoshihiro Fukuoka*, Tsugutomo Sakurada and Nobuyuki Hara

A 50-year-old man with Churg-Strauss syndrome showed granulocytes (GNLs) which generated more superoxide anion ($O_2^-$) than GNLs from healthy subjects following in vitro stimulation with C5a or C3a. Production of $O_2^-$ subsided as the clinical symptoms improved with steroid treatment. A hyperresponsiveness of GNLs may be involved in this disorder.

(Internal Medicine 34: 1005-1008, 1995)

Key words: anaphylatoxins, Churg-Strauss syndrome, superoxide anion, eosinophilia, granulocyte, complement

Introduction

Allergic granulomatosis and angitis, or Churg-Strauss syndrome, is a rare disorder of unknown etiology (1). Bronchial asthma is a typical early complaint. The findings of marked eosinophilia and elevation of serum IgE suggest that a hypersensitivity reaction is involved in the inflammatory reaction in this disorder (2). Complement activation products, C5a and C3a, are considered to be important pro-inflammatory mediators in acute inflammation. Since granulocytes (GNLs) are one of the most important target cells for the anaphylatoxins, C5a and C3a (3), we studied the manner of GNL response to C5a and C3a in the clinical course of a Churg-Strauss syndrome patient.

Case Report

Clinical and laboratory data

A 50-year-old Japanese man was admitted with the complaint of exertional dyspnea with wheezing for the previous 3 months. He had a 3-year history of pollinosis, but there was no history of bronchial asthma or drug allergy, and no family history of allergic disorders.

Examination of the chest revealed wheezing in the upper and middle lung fields bilaterally. Routine laboratory findings were within the normal range. The patient was diagnosed as having bronchial asthma, and was treated with an oral xanthine and steroid inhalation. On the fifth hospital day, he developed a high fever which persisted for the next 3 days. He also exhibited leukocytosis ($16,890/mm^3$), eosinophilia ($1,900/mm^3$) and an elevated C-reactive protein level (4.5 mg/dl). A chest X-ray revealed a slightly infiltrative shadow on the right lower lung field, and an antibiotic (cefotiam 1g×2/day; d.i.v.) was added to the regimen. The fever and abnormal shadow on the chest X-ray disappeared in a few days. The serum level of C-reactive protein returned to normal. However, as the eosinophilia worsened, under the suspicion that it was due to an allergic reaction, the antibiotic was discontinued. Nevertheless the eosinophilia progressed rapidly and the patient developed a generalized skin eruption. The wheezing and skin eruptions grew worse; intravenous infusion of a corticosteroid (methylprednisolone 250 mg×1/day; d.i.v.) were administered for the first days of the third week. He rapidly improved, but in one week he exhibited a relapse with marked eosinophilia (24,000/mm$^3$). His serum level of IgE also elevated (2,300 IU/ml). The levels of C3, C4, and CH50 were all within the normal range, however perinuclear antineutrophil cytoplasmic antibody pANCA was detected. He had a high fever, wheezing, a generalized skin eruption, and melena. Histological examination of biopsy specimens of the skin and intestinal mucosa showed infiltration of eosinophils beneath the basement with fibrinoid degeneration of the arterioles (Fig. 1). While we observed no obvious granulomatous lesions, a characteristic of Churg-Strauss syndrome, the patient’s clinical course, laboratory findings, and histological observations led to a diagnosis of that disease. Furthermore, the detection of pANCA helped to confirm the diagnosis of Churg-Strauss syndrome. One week after a sys-
Tanaka et al

Figure 1. Histological examination of skin (left) and intestine (right). Specimens of mucosa obtained at biopsy show eosinophilic infiltration below the basement membrane with fibrinoid degeneration of arterioles, but no obvious granuloma formation (HE stain, x400).

temic corticosteroid (prednisolone 1 mg/kg/day) was started, his symptoms disappeared and the eosinophil count and total IgE titer decreased. When the eosinophil count increased again, hydroxyurea (Hydrea 1,000 mg/day) was added to the medication.

Measurement of superoxide (O$_2^-$) production by GNLs

To investigate the influence of complement activation on GNLs we conducted studies of the GNLs production of O$_2^-$ following in vitro stimulation with C5a and C3a.

Preparation of GNLs.

Venous blood was drawn into heparinized syringes from the patient and from healthy subjects; all gave informed consent to this study. GNLs were isolated as previously reported (4). Briefly, after elimination of erythrocytes by dextran sedimentation followed by a brief hypotonic lysis, the cell suspension was centrifuged in LSM (lymphocyte separation medium; Organon Teknika, Durham, NC) gradient to separate the GNL from other cells. The number of GNLs was counted, and their viability was determined to be at least 90% in the presence of 0.1% trypan blue.

Production of superoxide anion (O$_2^-$) by GNL

O$_2^-$ was measured by the superoxide dismutase inhabitable ferricytochrome C reduction method with PMA (phorbol myristate acetate, Sigma, St. Louis, MO) (600 ng/ml), recombinant human C3a (5) (100 ng/ml), and purified human C5a (10 ng/ml) (donated by Dr. T.E. Hugli of The Scripps Research Institute, La Jolla, CA), as the triggers. In brief, the reaction mixtures (total volume = 1 ml) containing 2x10$^5$ GNL and 100 μM ferricytochrome C (type 4, Sigma) in the HEPES buffer were preincubated at 37°C for 7 minutes. Each stimulant was then added to the reaction mixture. Without the stimulation, no production of O$_2^-$ was observed. The change in absorbancy at 550–540 nm was followed on a recorder attached to a double-beam spectrophotometer (Hitachi 557, Tokyo). The release of O$_2^-$ was determined by the molar absorption coefficient for reduced-minus-oxidized ferricytochrome C of 19.1x10$^3$ M$^{-1}$xcm$^{-1}$ and by using the initial rate of the reaction.

The amount of O$_2^-$ produced by the patient’s GNLs following stimulation with C5a or C3a increased during the exacerbation of his symptoms: 5.97 nmol/min/2x10$^5$ cells with C5a, 2.93 with C3a, 4.82 with PMA, as compared with a control level of 0.13 with C5a, of 0.16 with C3a and of 1.53 with PMA (Fig. 2). The production of O$_2^-$ by C5a and C3a gradually decreased to that of normal subjects after beginning the administration of a systemic corticosteroid. The blood eosinophil count and IgE titer also decreased in parallel with the decrease in O$_2^-$ production by in vitro stimulation of GNLs. When the wheezing relapsed three months later, the amount of O$_2^-$ following stimulation of GNLs with C5a and PMA rose to high levels: 2.30 nmol/min/2x10$^5$ cells with C5a (control level = 0.31) and 5.65 with PMA (control level = 1.99). This exaggerated response to C5a and PMA declined to the control level after the disappearance of the wheezing, as shown in Fig. 2.

Discussion

Churg-Strauss syndrome is a rare disorder that is characterized by granulomatous angiitis, bronchial asthma and eosinophilia (1). Although the present patient did not exhibit granuloma formation in the biopsy specimens obtained from cutaneous, intestinal, and pulmonary lesions, we did observe fibrinoid degeneration of arterioles surrounded by eosinophils. Preexisting asthma, marked eosinophilia and pANCA detection lead to the diagnosis of Churg-Strauss syndrome.

The pathophysiological findings in previous cases (6, 7) suggested a hypersensitivity reaction to an unidentified antigen as the cause. The finding of marked eosinophilia and high serum titers of IgE in the present patient are consistent with this hypothesis. The presence of angiitis suggests the presence of the Arthus type reaction. It is therefore speculated that comple-
ment plays a role in the inflammatory reaction in this disorder. However, this patient’s serum complement activity (CH50) was within the normal range during the active stage of the disease, suggesting that no significant consumption of complement occurred. On the contrary, it is not known whether the responses to C5a and C3a in this patient’s GNLs are similar to those of normal subjects.

GNLs are among the most important target cells for the anaphylatoxins, C5a and C3a (3). These anaphylatoxins are produced following the activation of complement and act as triggers of such biological events as the release of chemical mediators, chemotaxis, and the aggregation of granulocytes (3, 8). We therefore studied the production of O$_2^-$ by this patient’s GNLs following stimulation with C3a and C5a. During the active stage, his GNLs produced a greater amount of O$_2^-$ than did the GNLs from control subjects. This increased production of O$_2^-$ gradually subsided as the clinical symptoms improved on the administration of a corticosteroid. As shown in Fig. 2, the increased production of O$_2^-$ appeared following stimulation with C5a during a relapse of symptoms.

In this study we used density gradient centrifugation to purify GNLs which were composed of neutrophilic and eosinophilic leukocytes. It has been reported that eosinophils produce O$_2^-$ following stimulation with C5a or C3a (9). Moreover, an eosinophil product (major basic protein) potentiates O$_2^-$ production from neutrophils (10). It has not yet been established whether such an upregulation of O$_2^-$ generation by GNLs may be attributed to the upregulation of eosinophil O$_2^-$ generation or to that of neutrophil O$_2^-$ generation influenced by the eosinophilic products. Since eosinophils purified by negative selection with anti-CD16 antibody showed higher O$_2^-$ production than the granulocytes (unpublished observation), the upregulation of eosinophil O$_2^-$ generation may be the most feasible explanation for these results. Nevertheless, it is suggested that the hyperresponsiveness of GNLs to C5a and C3a contributes to the acute inflammatory reaction in this disorder.

Acknowledgements: We thank Dr. T.E. Hugli (Department of Immunology, The Scripps Research Institute) for donating purified human C5a, Dr. S. Hashimoto and Dr. M. Nakamura (1st Dept. of Internal Medicine, Kyushu University) for their comments on histological studies and M. Suga for her technical assistance.

References

5) Fukuoka Y, Yasui A, Tachibana T. Active recombinant C3a of human anaphylatoxin produced in Escherichia coli. Biochem Biophys Res


