Cell-bound and Circulating IgE Antibody to Herpes Simplex Virus

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SUMMARY

Mice immunized with ultraviolet-inactivated herpes simplex virus (HSV) or injected with infectious virus developed IgE-specific antibody to HSV. Cell-bound IgE was detected by measuring the release of histamine from peritoneal mast cells challenged in vitro with virus antigens. Circulating IgE antibody was detected by sensitizing rat basophilic leukaemia (RBL-2H3) cells with sera from HSV-immunized mice and then challenging these cells with virus or control antigens to release histamine. IgE antibody may contribute to the pathogenesis of virus infections.

INTRODUCTION

The immune response to virus challenge can limit the infection but in some cases results in immunological injury (Notkins & Koprowski, 1973). Such injury could be due to mediators released from mast cells or basophils. This could occur directly as a result of virus-induced cytopathology or indirectly through the secretory stimulation of mast cells by virus antigens reacting with cell-bound IgE. The humoral immune response following natural infection or immunization with a virus results in predominantly IgM, IgG and IgA antibodies directed against the virus (Ogra et al., 1975). There is little information on anti-virus IgE antibodies. However, a relationship between increased total serum IgE levels and virus infections has been observed in several studies. During infectious mononucleosis, total IgE serum levels are elevated, but return to pre-illness levels by 3 months (Bahna et al., 1978). In a retrospective study, the total serum IgE levels were higher during acute virus infections than several weeks later (Perelmutter et al., 1979), and in a prospective study of children of atopic parents, the development of specific IgE against allergens appeared after virus respiratory infections (Frick et al., 1979). Thus, certain virus infections, presumably by influencing suppressor cell networks, can enhance the production of IgE antibodies.

Specific IgE antibodies against herpes simplex virus (HSV) types 1 and 2, in rabbits, were reported by Day et al. (1976). Recently, anti-respiratory syncytial virus IgE antibodies were found in children after infection with this virus (Welliver et al., 1981). In this paper, we describe a mouse model for the development of IgE anti-virus antibodies. IgE anti-HSV antibodies developed after either immunization with ultraviolet (u.v.)-inactivated virus or natural infection.

METHODS

Virus. HSV 1, F strain, was propagated in human amniotic WISH cells and purified as described by Spear & Roizman (1972). In brief, pellets from infected and uninfected cells were processed by rate zonal centrifugation on a CsCl-sucrose linear gradient and both virions (1.21 to 1.26 g/ml) and nucleocapsids (1.29 to 1.35 g/ml) collected. The protein concentration in the virion-enriched and nucleocapsid-enriched preparations was 250 μg/ml and 130 μg/ml, respectively. Control cell preparations were processed in the same manner and identical fractions collected (protein concentration < 10 μg/ml). Samples were subsequently diluted to give equivalent concentrations of protein.

Immunization. Six- to eight-week-old male A/J mice were injected subcutaneously with cyclophosphamide (Sigma) at a dose of 100 μg per gram body weight 3 days before immunization. Cyclophosphamide inhibits T
suppressor cells and enhances IgE production (Ishizaka, 1976). One to 3 μg of u.v.-inactivated HSV nucleocapsids or virions (i.e. between $3 \times 10^6$ and $1 \times 10^7$ p.f.u.), mixed with 0.5 to 1.0 mg of aluminium hydroxide in 0.5 ml saline, was injected subcutaneously in the back. U.v.-inactivation was carried out for 1 min with a 30 W GE germicidal lamp (G 15T8) placed 15 cm from the virus suspension. After treatment, infectious virus could not be detected ($< 10^2$ p.f.u./ml). Mice were immunized in the same way with an equivalent concentration of control antigen and, at different times thereafter, animals were sacrificed and peritoneal cells collected. The cells were washed three times with cold PIPES-buffered medium (119 mM-NaCl, 5 mM-KCl, 25 mM-PIPES, 5-6 mM-glucose and 0.03% human serum albumin, pH adjusted to 7/4 with NaOH) and resuspended in PIPES medium containing 1.0 mM-Ca$^{2+}$ and 0.5 mM-Mg$^{2+}$ (PIPES ACM). Usually, 1 x 10$^6$ to 3 x 10$^6$ cells were obtained per mouse; 3 to 5% of total cells were mast cells, identified by toluidine blue staining. The cells studied in each experiment were pooled from 2 or 3 mice. Under these conditions, histamine release is due only to IgE antibodies (Siraganian & Hazard, 1979; Fox et al., 1982).

**Histamine assay.** The histamine release assay was performed as described previously (Siraganian & Hazard, 1979). In brief, 0.2 ml (2-0 x 10$^5$ p.f.u.) of serially diluted HSV virions or control cell antigens in PIPES ACM were mixed with 0.1 ml of 5 μg/ml α-L-phosphatidyl-L-serine in PIPES ACM (Analabs Inc., North Haven, Ct., U.S.A.) and 0.2 ml of peritoneal cells (1.0 ± 0.5 x 10$^5$ cells/tube) were added. After incubation at 37 °C for 30 min, the tubes were centrifuged and the supernatant fluids were collected and assayed for histamine by the automated fluorimetric technique (Siraganian & Hook, 1980). Total histamine content in each tube ranged from 40 to 260 ng/ml.

**Assay of IgE antibodies.** Circulating IgE antibody was measured by its capacity to sensitize passively basophils for antigen-induced histamine release. A rat basophilic leukaemia cell line (RBL-2H3) has IgE receptors and degranulates non-cytotoxically through IgE-mediated reactions (Siraganian et al., 1982). Histamine release is due to IgE antibodies, as washing of the cells removes the IgE immunoglobulins which have much lower affinity for their cell surface receptors (Segal et al., 1981). The 2H3 cloned subline was maintained as described previously (Barsumian et al., 1981). Cells were seeded in 96-well microplates in RPMI 1640 media supplemented with 10% foetal bovine serum. Each well, containing 2 x 10$^4$ to 5 x 10$^4$ cells, was incubated at 37 °C in a 5% CO$_2$ atmosphere for 24 h. The wells were washed three times with PIPES ACM, and 0.1 ml of 1:5 dilution of the serum sample was added. After 1 h of incubation, the serum sample was removed by washing the cells with PIPES ACM. The sensitized cells then were incubated with 0.05 ml of HSV virions (5 x 10$^7$ p.f.u.) diluted 1:20, which normally gave the highest release of histamine. After 30 min of incubation at 37 °C, 0.05 ml of supernatant fluids were removed and assayed for histamine. The total histamine content of representative wells was determined by adding 2% perchloric acid to lyse the cells. The percent histamine release was calculated as described previously (Siraganian & Hook, 1980).

**RESULTS**

Mice were immunized with u.v.-inactivated HSV and 28 days later peritoneal cells were harvested and challenged with virus or control cell antigens. The data in Fig. 1 (a) show that cells from HSV-immunized mice challenged with HSV released up to 75% histamine, whereas cells challenged with control antigens released less than 5% histamine. Cells from mice immunized with control cell antigens failed to release histamine when exposed to either HSV or control cell antigens.

The time after immunization at which the peritoneal cells became sensitive to challenge with HSV is illustrated in Fig. 1 (b). Cells obtained 8 days after immunization failed to release histamine, whereas cells obtained at 14, 21, and 42 days released significant amounts. The release curve was still rising at a 10$^{-1}$ dilution of HSV. In other experiments, animals were boosted with HSV 21 days after primary immunization. In contrast to cells from animals receiving only one injection of virus (Fig. 1 b), cells from animals receiving a booster (Fig. 1 c) released more histamine at a lower concentration of challenge antigen (i.e. the curves show a shift to the left).

Evidence that IgE antibody to HSV was present also in the circulation of immunized mice was obtained by sensitizing RBL-2H3 histamine-releasing cells. The RBL-2H3 cells were incubated with sera obtained from mice at different days after immunization and then challenged in vitro with HSV or control cell antigens. As seen in Fig. 2, specific IgE antibody was not detected until 14 days after primary immunization. Sera from animals boosted with HSV on day 21 released even more histamine, and specific IgE antibody was detected until the termination of the
IgE antibody to HSV

Fig. 1. Histamine release from peritoneal cells of mice immunized with HSV. (a) Mice were immunized with u.v.-inactivated HSV (●) or an equivalent concentration of control cell antigens (○). After 28 days, peritoneal cells were removed and challenged with different concentrations of HSV (——) or control cell antigens (——) as described in Methods. (b) Mice were immunized with u.v.-inactivated HSV and at 8 (○), 14 (▲), 21 (●) and 42 (■) days after immunization, peritoneal cells were collected and challenged with different concentrations of HSV. (c) Mice were immunized with u.v.-inactivated HSV, but boosted with antigen 21 days after the primary immunization. Peritoneal cells were collected and challenged with HSV 15 (●), 31 (▲) and 63 (■) days after the booster. (d) Mice were pretreated (——) or not pretreated (—–) with cyclophosphamide and then inoculated with 2 × 10^4 p.f.u. of HSV (closed symbols) or an equivalent dilution of control cell antigens (open symbols). On days 14 (circles) and 21 (triangles), peritoneal cells were harvested and challenged with different concentrations of HSV.

experiment on day 140. Cells sensitized with sera from HSV-immunized animals failed to release histamine when challenged with control cell antigen. Serum from mice immunized with control cell antigens (control serum) failed to sensitize RBL-2H3 cells, as measured by the lack of histamine release following exposure to either HSV antigens or control cell antigens. In these experiments, anti-mouse IgE, which released 60 to 65% histamine, served as a positive control.

Evidence that a natural infection with live virus also can induce IgE-specific antibody was obtained by infecting mice with 2 × 10^4 p.f.u. of HSV subcutaneously on the back. This route of inoculation produces a mild local infection. The data in Fig. 1(d) show that peritoneal cells obtained 14 and 21 days after infection released significant amounts of histamine when exposed to HSV. Higher values were obtained when mice were pretreated with cyclophosphamide.
Fig. 2. IgE antibody to HSV in serum of immunized mice. Mice were immunized with 1 μg of u.v.-inactivated HSV (Ag) on day zero and boosted with the same concentration of antigen on day 21. On different days serum samples were collected, diluted 1:5 and incubated with RBL-2H3 cells. After washing, the cells were challenged with a 1:20 dilution of either HSV (■) or control cell antigens (□) and histamine release measured. Control serum was obtained from mice immunized with control cell antigens.

DISCUSSION

These experiments show that IgE anti-virus antibodies develop in mice given u.v.-inactivated or infectious HSV. The IgE antibodies appeared within 14 days after immunization and persisted (after a second injection of virus) for at least 4 months. Methods of immunization were selected which favoured the formation of IgE antibodies (Siraganian, 1981). Both the route of immunization and the antigen concentration are critical in promoting specific IgE production. Injection was subcutaneous with low concentrations of the u.v.-inactivated virus in aluminium hydroxide. Treatment with cyclophosphamide is known to depress the activity of suppressor T cells and enhance IgE responses (Ishizaka, 1976). In our experiments, the IgE anti-HSV response was greater in the cyclophosphamide-treated mice (Fig. 1d). This suggests that anti-virus IgE responses might be particularly prominent in conditions where there is a decrease in suppressor T cells, e.g. in congenital immunodeficient states or secondary to immunosuppressive therapy.

IgE antibodies bind avidly to mast cells and basophils. This characteristic was utilized in the two assays for IgE antibody. In the first system, histamine was released from mast cells of immunized or infected mice on incubation with virus proteins. This is a measure of the presence of IgE already on mast cells. In the second system, sera from these mice passively sensitized RBL-2H3 cells for antigen-triggering and histamine release.

Virus infections and the induction of IgE antibodies might be involved in certain disease processes. For example, an association between virus respiratory infections and exacerbation of asthma has been observed (Berkovich et al., 1970; McIntosh et al., 1973; Minor et al., 1974; Empey et al., 1976). Viruses might increase mucosal secretions and/or act on bronchial receptors. Previously, we found that virus and immune interferon enhanced IgE-mediated histamine release from human basophils (Ida et al., 1977, 1980) and this required the synthesis of new RNA (Hernandez-Asensio et al., 1979). The present experiments demonstrate another mechanism by which viruses could modulate mediator release, i.e. the direct interaction of virus proteins with virus-specific IgE. The triggering of mast cells results in the release of histamine, leukotrienes, chemotactic and other inflammatory factors. These factors could localize an infection and might play a role in a variety of disease processes, including the bronchial constriction observed with certain virus infections. The capacity of other viruses to induce specific IgE antibodies and the role of these antibodies in immunological defence and injury merit further investigation.
IgE antibody to HSV

REFERENCES


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