Induction of an Organ-Specific Autoimmune Disease, Lymphocytic Hypophysitis, in Hamsters by Recombinant Rubella Virus Glycoprotein and Prevention of Disease by Neonatal Thymectomy

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Glycosylated, membrane-associated E1 (58-kDa) and E2 (47- to 49-kDa) rubella virus proteins and unglycosylated nucleoprotein C (33 kDa), from separately expressed vaccinia virus recombinants, were injected into golden Syrian hamsters. Rubella virus E1 and E2 glycoproteins consistently induced an organ-specific autoimmune disease, autoimmune lymphocytic hypophysitis, which was evidenced by the induction of autoantibodies against pituitary cells and by lymphocytic infiltration of the pituitary. Neonatal thymectomy prevented the disease. In contrast, rubella virus nucleoprotein C did not induce either autoantibodies against pituitary cells or lymphocytic infiltration of the pituitary. This finding raises the possibility that virus-specific protein itself can induce an organ-specific autoimmune disease in certain circumstances.

It is generally believed that autoimmune diseases result from a combination of exogenous (viruses, drugs, and toxins) and endogenous (genes and hormones) factors. Viruses have long been suggested as an exogenous factor which may trigger autoimmunity and have been increasingly implicated in the etiology of such disease (13, 15, 27). However, there is little information as to whether any particular viral protein itself can induce autoimmune disease. Previous reports have demonstrated that an autoimmune disease, experimental allergic encephalomyelitis, can be induced in mice and rats by injection of encephalitogenic proteins such as myelin basic protein (MBP), proteolipoprotein, or encephalitogenic peptides (5, 16, 30). In this study, we investigated whether the rubella virus structural proteins can induce an organ-specific autoimmune disease in golden Syrian hamsters.

Rubella virus structural proteins are encoded by a 24S subgenomic RNA, which is translated into a 110-kDa polyprotein precursor and is posttranslationally processed into the structural proteins C, E2, and E1, in that order (10, 14). E1 (58 kDa) and E2 (47 to 49 kDa) are glycosylated, membrane-associated proteins, and C (33 kDa) is an unglycosylated nucleoprotein (7). We constructed vaccinia virus recombinants (pE1, pE2, and pC) which express separately the individual rubella virus structural proteins (Fig. 1). The expressed recombinant rubella virus E1, E2, and C proteins were isolated from CV-1 cells infected with the constructed vaccinia virus recombinants (pE1, pE2, or pC) (11). Briefly, CV-1 cells infected with the recombinant vaccinia viruses (pE1, pE2, or pC) were lysed in RIPA buffer (50 mM Tris-HCl [pH 7.4], 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate [SDS], 150 mM NaCl) containing 1% Nonidet P-40. The cellular lysates were then electrophoresed on SDS-10% polyacrylamide gels, and the appropriate bands were electroeluted as described elsewhere (11) and extensively dialyzed in 10 mM Tris buffer (pH 7.4) before injection into hamsters. The antigenicities of the expressed proteins from the vaccinia virus recombinants were found to be similar to those of the authentic viral antigens of rubella virus.

Four-week-old male LGV strain golden Syrian hamsters (17 to 21 per group) were inoculated intradermally with E1, E2, or C protein (100 μg in 0.1 ml of 10 mM Tris buffer [pH 7.4] per animal) three times at weekly intervals. Fifteen control animals were injected with 0.1 ml of 10 mM Tris buffer (pH 7.4). Blood was obtained from the retroorbital plexus of each animal prior to injection and each week after the first injection for 11 weeks; serum was collected from each blood sample (28). To determine whether autoantibodies were present, frozen sections of pancreas and of pituitary, thyroid, and adrenal glands from healthy hamsters (4 to 6 weeks old) were incubated with 20 μl of each sample serum (1:8 dilution in phosphate-buffered saline [PBS]) in a humid chamber at room temperature for 2 h. After three washes with PBS, 20 μl of fluorescein isothiocyanate-labeled goat anti-hamster immunoglobulin G (1:20 dilution in 0.01% Evans blue and PBS) (Cedarlane Laboratories, Hornby, Ontario, Canada) was added to the sections, which were then kept in a darkroom for 2 h at room temperature. After an additional washing with PBS, the sections were examined under a fluorescence microscope (29).

Two weeks after injection with rubella virus E1 glycoprotein, 50% of the hamsters had developed autoantibodies against pituitary cells (Fig. 2b). Three weeks after injection, autoantibodies were found in 95% of the animals. This level was constant for 1 week but started to decline gradually after that. At 8 weeks, only 20% of the hamsters still had antibodies against pituitary cells. The appearance of autoantibodies against pituitary cells in animals injected with rubella virus E2 glycoprotein was delayed approximately 2 weeks but followed a trend similar to that seen in the animals injected with E1. In contrast, none of the animals that received unglycosylated nucleoprotein (C protein) developed autoantibodies (Table 1) (Fig. 2a). Furthermore, none
of these proteins (E1, E2, or C) induced autoantibodies against thyroid, adrenal cortex, or islet cells. Control animals that received only Tris buffer did not develop autoantibodies against pituitary cells or against cells of other tissues tested. Eight weeks after the final injection of the recombinant rubella virus proteins, all animals were sacrificed and sections of pituitary, thyroid, adrenal cortex, and pancreas from the experimental and control animals were examined microscopically after being stained with hematoxylin and eosin. All of the animals that developed antibodies against pituitary cells by injection with E1 or E2 glycoprotein showed diffuse inflammatory cell infiltration throughout the pituitary gland (Fig. 3b), a condition comparable to lymphocytic hypophysitis in humans. In addition, the three animals which did not develop autoantibodies against pituitary cells did, however, show inflammatory lesions in the pituitary gland. In contrast, none of the control animals showed lymphocytic infiltration of the pituitary gland. None of the animals that had received nonglycosylated nucleoprotein (C protein) showed such lesions in the pituitary (Fig. 3a). Furthermore, none of the animals showed inflammatory lesions in the thyroid, adrenal cortex, or pancreas. When additional 12 hamsters were immunized with vaccinia virus (nonrecombinant)-infected CV-1 cell lysates as controls, none of the animals developed antibodies against pituitary cells or lymphocytic infiltration of the pituitary gland. In contrast, all six hamsters infected with rubella virus (wild type, M33 strain) developed autoimmune lymphocytic hypophysitis, as evidenced by the induction of autoantibodies against pituitary cells and lymphocytic infiltration of the pituitary.

Prior to sacrifice of hamsters at 8 weeks after the final injection of the recombinant rubella virus proteins, all animals were weighed. The mean body weight of E1- or E2-treated hamsters (E1, 96.14 ± 12.92 g [x ± standard deviation]; E2, 97.02 ± 13.21 g) was significantly lower (P < 0.01) than that of the 10 untreated control animals (130.21 ± 5.43 g) or the C-protein-treated hamsters (128.94 ± 4.95 g).

Twelve neonatal thymectomized hamsters were injected with E1, and 12 others were injected with E2. Nine of the 12 animals receiving E1 showed neither production of antibodies against pituitary cells nor lymphocytic infiltration of pituitary cells (Table 1). Similarly, of the 12 animals receiving E2, 10 did not show antibody production or lymphocytic infiltration in the pituitary throughout the course of the experiment (Table 1). In contrast, most of the thymectomized hamsters that received either E1 or E2 showed diffuse lymphocytic infiltration throughout the pituitary (Table 1). These results suggest that T cells play a crucial role in the development of an autoimmune lymphocytic hypophysitis-like syndrome. When C proteins were injected into neonatal thymectomized hamsters, none of the animals developed antibodies against pituitary cells or inflammatory lesions of the pituitary. The results were the same for normal (i.e., thymectomized) hamsters.

The first description of autoimmune destruction of the pituitary gland appeared in the medical literature in 1962 (6), since which time at least 28 histologically documented


from dissimilar genes or by their protein products (15, 22) An antigenic determinant on rubella virus glycoproteins E1 and E2 might be similar to a determinant on the pituitary cells. Amino acid sequence comparison of E1, E2, and C proteins showed that E1 and E2, but not C, share a common sequence of three alanines, at positions 442 through 444 (Ala-Ala-Ala) of E1 and positions 113 through 115 (Ala-Ala-Ala) of E2 (2). The three alanines of the E2 peptide have been localized near the sequence of the immunodominant helper-T-cell antigenic site (12). Whether the alanine sequence of E1 and E2 proteins plays a role in the triggering of T-cell-mediated organ-specific autoimmune lymphocytic hypophysitis remains to be determined. In addition, whether the same amino acid sequence is present in the protein from the antigenic domain of pituitary cells is not known. If the specific amino acid sequence is present in the pituitary cells antibodies raised against the antigenic determinant of the rubella virus E1 and E2 glycoproteins could react with pituitary cells. A recent study of ours showed that islet cell antibodies that reacts with a 38-kDa human pancreatic islet cell-specific protein can be induced by human cytomegalovirus (17), possibly because of similar epitopes shared by islet cell-specific proteins and antigenic determinants of cytomegalovirus (15). It was also shown that amino acid sequence homology exists between viral (hepatitis B virus proteins and the encephalitic antigen of rabbit MBP (4) Hepatitis B virus polymerase (HBVP) was found to share such consecutive amino acids with the encephalitic site of rabbit MBP. Rabbits given injections of a selected 8-0 10-amino-acid peptide from HBVP produced antibodies that reacted with the sequences of HBVP and also with native MBP. It is well-known that self-mimicking epitopes of foreign invading agents such as bacteria are capable of triggering vigorous autoimmune responses (18). Several studies have shown that antigenic similarity or molecular mimicry between antigens of infectious agents and host tissue may explain the induction of autoimmunity by microbes (23, 25). Whether there are antigenic similarities between E1 and E2 glycoproteins and antigens on the pituitary cells remains to be determined.

What role do autoantibodies against pituitary cells play in the pathogenesis of autoimmune lymphocytic hypophysitis? Since autoantibodies are believed to play a role in the pathogenesis of several diseases (24, 26), we experimented to see whether these autoantibodies could induce such autoimmune disease. We failed to produce autoimmune hypophysitis by passive transfer of the antibodies, suggesting that the presence of the autoantibodies may be an epiphenomenon rather than an indication of their involvement in target cell destruction. Some earlier report indicating that high titers of autoantibodies can be detected in healthy family members of patients with autoimmune diseases support our observation (8, 19). It is apparent that neither a high titer of autoantibodies nor simply their presence reflects the development of autoimmune disease.

What pathogenic mechanism could explain the induction of lymphocytic hypophysitis by E1 and E2 proteins? Our experimental data revealed that neonatal thymectomy prevented lymphocytic hypophysitis. Thus, there is a possibility that the induction of the disease by multiple injections with recombinant E1 and E2 proteins of rubella virus is due to an antigen-specific T-cell-mediated autoimmune mechanism this mechanism may be similar to that in which MBP can induce autoimmune experimental allergic encephalomyelitis in genetically susceptible animals (30). Similarly, T-cell mediated autoimmune disease has also been found in the

### Table 1. Effect of thymectomy on the development of autoimmune lymphocytic hypophysitis

<table>
<thead>
<tr>
<th>Thymectomy</th>
<th>Immunizing protein</th>
<th>No. of hamsters with autoantibody against pituitary cells (total) no. inculated (%)</th>
<th>No. of hamsters with lymphocytic infiltration of pituitary (total) no. inculated (%)</th>
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<tr>
<td>No</td>
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<td>E2</td>
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</tr>
<tr>
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<td>5/12 (42)</td>
<td>5/12 (42)</td>
</tr>
<tr>
<td></td>
<td>E2</td>
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</tr>
<tr>
<td></td>
<td>C</td>
<td>0/12 (0)</td>
<td>0/12 (0)</td>
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* Hamsters were thymectomized 2 to 3 days after birth. P \(<\) 0.001 between thymectomized group (E1 and E2) and nonthymectomized group (E1 and E2) for incidence of autoantibody and lymphocytic infiltration.

* Pathological changes in the pituitary gland were examined 8 weeks after immunization with E1, E2, or C protein.
FIG. 3. Pathological changes in the pituitary gland of hamsters 8 weeks after injection with recombinant rubella virus E1 glycoprotein. Sections of pituitary from hamsters injected with rubella virus nonglycosylated nucleoprotein C (a) or from uninfected age-matched controls were examined. The pituitary shows no sign of microscopic changes (single arrows indicate normal pituitary cells). Sections of pituitary from hamsters injected with rubella virus glycoprotein E1 (b) were examined. Pituitary shows degeneration and necrosis with infiltration by inflammatory cells (double arrows indicate inflammatory cell infiltration). Magnification, ×200.

model of experimental autoimmune uveitis (20). Rubella virus glycoprotein-induced lymphocytic hypophysitis and MBP-induced experimental allergic encephalomyelitis share some common immunologic features, since both diseases can be prevented by neonatal thymectomy. However, T-cell transfer experiments remain to be conducted to define the specific role of T cells in the pathogenesis of autoimmune lymphocytic hypophysitis induced by rubella virus glycoproteins in hamsters.

In this study, we have demonstrated for the first time that an autoimmune lymphocytic hypophysitis-like syndrome can be experimentally induced by specific viral proteins,
such as the recombinant glycosylated, membrane-associated proteins (E1 and E2) of rubella virus, in golden Syrian hamsters and that the disease can be prevented by neonatal thymectomy. This animal model may be invaluable in studying human autoimmune lymphocytic hypophysitis and in elucidating the molecular and immunological role of virus-specific proteins in the initiation of organ-specific autoimmune diseases.

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REFERENCES


