Cell Receptors for the Mammalian Reovirus: Reovirus-Specific T-Cell Hybridomas Can Become Persistently Infected and Undergo Autoimmune Stimulation

NOBORU MATSUZAKI,1,2 VIRGINIA S. HINSHAW,3 BERNARD N. FIELDS,1 AND MARK I. GREENE4*

Departments of Pathology, Microbiology and Molecular Genetics, and Medicine (Infection Disease), Harvard Medical School and Brigham and Women's Hospital, and the Shipley Institute of Medicine, Boston, Massachusetts 02115; School of Medicine, Tufts Medical School, Boston, Massachusetts 02111; University of Wisconsin, Madison, Wisconsin 53715; Division of Immunology, Department of Pathology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received 11 April 1986/Accepted 26 June 1986

We have previously described the development of virus-specific helper T cell hybridomas which recognize structural determinants shared by type 1 and type 3 reoviruses that have been exposed to UV radiation. We have found that T-cell hybridomas become persistently infected with live type 3 reovirus used for the immunization. Persistently infected T-hybridoma cells were found to spontaneously produce interleukin 2 (IL-2). To analyze the mechanism of induction of IL-2 secretion of persistently infected T-cell hybridomas, we exposed T-cell hybridomas specific for UV-treated virus to replicating type 3 reovirus. The T-cell hybridomas became infected but did not produce IL-2 unless simultaneously exposed to syngeneic I-A antigens-presenting cells. In this situation, the persistently infected T-cell hybridomas produced IL-2 without being reexposed to virus. This process was not a consequence of nonspecific IL-2 gene activation, which occurs in cells persistently infected with reovirus, because reovirus infection did not activate IL-2 secretion in T-cell hybridomas with other antigenic specificities. Reovirus exposure also resulted in persistent infection of certain antigen-presenting B-cell tumor lines. The persistently infected B-cell tumor lines could stimulate reovirus-specific helper T cells but not T-cell hybridomas of other specificities. The data support the thesis that persistent infection of reovirus-specific T cells creates a mechanism in which the virus released from these cells is processed and then reexpressed by I-A antigens-presenting cells. The I-A antigen and reovirus structures on the antigen-presenting cells then restimulate the T cells through their specific receptors, resulting in IL-2 synthesis and release. These observations may be relevant to mechanisms of autoimmunity induced by virus.

Immunologic interactions between host cells and viruses have been investigated extensively and have provided insights into the role of T-cell immunity in host defense against viral infection (6, 25). In addition, studies with viruses have helped to clarify the major histocompatibility complex-induced restriction of T-cell recognition of foreign antigens on target cells (48).

Studies with the mammalian reoviruses have identified important genetic determinants involved in viral virulence (15, 27, 36). The mammalian reoviruses are segmented, double-stranded RNA viruses which have three outer capsid proteins (u1C [1, 4]) that play distinct roles in viral virulence (36). One of these proteins, the sigma 1 protein (hemagglutinin) encoded in the S1 RNA segment of the virus, has been shown to be the cell attachment protein (22, 44). One antigenic domain of the reoviral hemagglutinin identified by the neutralizing monoclonal antibody (9B5G) (7, 44), determines several biological interactions between reovirus and its cell surface receptor. Attachment and entry of virus can be blocked partially by the monoclonal anti-receptor antibody, 87.92.6 (21), which has been raised against the antigen-binding site of the 9B5 neutralizing monoclonal antibody (27, 28). The neutralization domain also provides a target for a significant proportion of the cytotoxic T-cell responses to reovirus (16, 28). The antibody can block a significant proportion of the reovirus-specific cytolytic activity (14, 28, 29).

Reovirus can infect some transformed lines without lysing the cells, resulting in persistent infection (2). L cells that are persistently infected with reoviruses show identifiable morphological changes (1). Such cells have altered cytoskeletons and contain a large number of lysosomelike structures in the cytoplasm. Reoviruses which persistently infect L cells show gradual changes in the hemagglutinin gene. The hemagglutinin protein is readily identifiable on the cell surface of the persistently infected cells. These persistently infected cells are also suitable targets for reovirus-specific cytolytic cells (36).

In a previous study (V. H. Hinshaw, N. Matsuaki, B. N. Fields, D. Furlong, and M. I. Greene, submitted for publication), we reported the development and the immunological characteristics of several reovirus-specific helper T-cell hybridomas induced with UV irradiation-treated type 3 reovirus. The T-cell hybridomas recognized epitopes of type 3 reovirus that became exposed after UV irradiation and which were shared with UV-irradiated type 1 reovirus. UV irradiation of reovirus was found to affect the outer capsid, leading to the complete loss of the sigma 1 protein. The reactivity to non-sigma 1 shared epitopes after immunization with UV-irradiated virus distinguishes the response from specific immune responses evoked by live virus.

In the present study, we immunized and fused live reovirus-primed T cells with BW5147 thymoma cells to obtain T-cell hybridomas specific for reovirus type 3. After exposure to live virus, the hybridoma cells spontaneously produced IL-2 as a consequence of developing persistent infection. To explore the mechanism of autostimulation of
T-cell hybridomas, we intentionally infected the specific T-cell hybridomas with reovirus. We observed that type 3 reovirus can persistently infect T-cell hybridomas and also certain antigen-presenting cell lines. We also determined that reovirus type 3 is released from the persistently infected T-cell hybridoma and is bound and then processed by I-A<sup>+</sup>-antigen-presenting cells and thereafter presented to the persistently infected reovirus type 3-specific T-cell hybridomas. Recognition of the I-A-reovirus antigenic complex antigen-presenting cell results in IL-2 production and secretion. The relevance of these observations to autoimmunity after virus infections is discussed.

MATERIALS AND METHODS

Animals. Female BALB/c mice were purchased from the Jackson Laboratories, Bar Harbor, Maine, and were maintained on standard lab chow and water ad libitum.

Virus. The origins of reovirus serotype 1 (Lang) and serotype 3 (Dearing) have been described previously (32). All viruses used in this study were purified from infected L-cell extract by procedures described previously (11, 39).

Cells. The development of T-cell hybridomas specific for UV-treated type 3 reovirus has been described elsewhere (Hinshaw et al., submitted). T-cell hybridomas induced by live reovirus immunization were established by the general procedure described by Kapppler et al. (20). BALB/c mice were immunized with intraperitoneal injection of 10<sup>5</sup> PFU of live reovirus type 3. Spleens were removed 1 week after the immunization and stimulated in vitro with reovirus-infected, irradiated (2,000 rad) syngenic spleen cells. After 4 days, viable cells were isolated on Ficoll-Hypaque gradients and fused with BW 5147 thymoma cells by using polyethylene glycol (molecular weight, 330 to 1,600; American Type Culture Collection, Rockville, Md.). The fused cells were cultured first in hypoxanthine-aminopterin-thymidine medium, next in hypoxanthine-thymidine medium, and then in medium used in the laboratory for T-cell hybridoma propagation (RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 10% NCTC 109 medium [M.A. Bioproducts], 4 mM l-glutamine, 1% antibiotics-antimycotic mixture [penicillin, 10,000 IU/ml; streptomycin, 10,000 IU/ml; fungizone, 25 μg/ml; GIBCO Laboratories, Grand Island, N.Y.], 15 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid], and 5 × 10<sup>-5</sup> M 2-mercaptoethanol). Hybridomas demonstrating positive responses to reovirus were cloned by limiting dilution. A variety of control T-cell hybridomas and 2A1 (keyhole limpet hemocyanin [KLH] specific) and A2.2B2 (hen egg white lysozyme [HEL] specific), were kindly provided by L. H. Glimerch. TA-3, an antigen-presenting B-cell hybridoma, was also a gift of L. H. Glimerch (17, 19). TA-3 was maintained in RPMI 1640 supplemented with 10% fetal calf serum, 4 mM l-glutamine, 15 mM HEPES, 1% antibiotics-antimycotic mixture, and 5 × 10<sup>-5</sup> M 2-mercaptoethanol.

Antigen. KLH (500 μg/ml) and HEL (100 μg/ml) were provided by M. Slauo. Purified type 1 and type 3 reoviruses were used at 10<sup>5</sup> PFU/ml.

Stimulation of IL-2 production. T-cell hybridoma cells (10<sup>5</sup>) and TA-3 cells (10<sup>5</sup>) were placed with the optimum concentration of antigen in 200-μl cultures. Supernatant fluids from these cultures were harvested at 24 h, and the level of IL-2 was assayed as described previously (17, 19).

IL-2 assay. IL-2 was assayed as described in previous studies (17, 19). Briefly, the culture supernatants were tested for the presence of IL-2 in the microassay by an IL-2-dependent helper T-cell line, HT-2, originally obtained by J. Watson (43). Cells (4 × 10<sup>5</sup>) were cultured for 24 h in the presence of 50% culture supernatant, and the degree of stimulation was measured by incorporation of [3H]thymidine (1 μCi/ml; New England Nuclear Corp. Boston, Mass.) into DNA after 20 h.

Infection of T-cell hybridomas or TA-3 B-cell hybridomas with reovirus. Infected T- or B-cell hybridomas were prepared by incubation with virus at a multiplicity of infection of 5 PFU per cell. After 1 h of incubation to allow virus to adsorb to the cells, the cells were added back for further culture.

Fixation of antigen-presenting B-cell hybridomas. Fixation was carried out with paraformaldehyde under conditions similar to those described by Chestnut et al. (5, 8) and by Ziegler and Unanue (46). Briefly, B-cell hybridomas were suspended at 5 × 10<sup>5</sup>/ml in balanced salt solution containing 0.5% freshly prepared paraformaldehyde. The cells were then incubated for 20 min at room temperature, and fixation was stopped by addition of cold balanced salt solution–10% fetal calf serum. The cells were pelleted by centrifugation and washed three times in balanced salt solution–fetal calf serum before being placed into culture.

Treatment of antigen-presenting B-cell hybridomas with chloroquine. Chloroquine was kindly provided by P. M. Allen, Harvard Medical School. To pulse B-cell hybridomas in the presence of 0.1 mM chloroquine, 5 × 10<sup>6</sup> cells were incubated in medium containing the drug for 30 min at 37°C. The cells were pelleted by centrifugation and suspended in medium containing the drug along with the antigen. After incubation for 2 h at 37°C, the cells were washed extensively, and then subjected with 0.5% paraformaldehyde (5, 8, 47).

RESULTS

Reactivity patterns of T-cell hybridomas after live type 3 reovirus immunization. In a previous study (Hinshaw et al., submitted), we showed that T-cell hybridomas induced with UV-treated reovirus type 3 were specific for determinants other than those expressed on the hemagglutinin. We demonstrated that UV treatment of type 3 reovirus destroys the sigma 1 protein (viral hemagglutinin) which determines the cellular and neural tropism of the reovirus serotype 3 (40, 44).

To analyze T helper cells specific for sigma 1, we immunized BALB/c mice with live type 3 reovirus. T-cell hybridomas generated after reovirus type 3 immunization demonstrated the reactivity pattern shown in Table 1. During screening, T-cell hybridomas which showed positive responses to type 3 reovirus could be categorized into two groups. T-cell hybridomas in group 1, exemplified by the

<table>
<thead>
<tr>
<th>Hybridoma</th>
<th>Proliferative response (cpm) against:</th>
<th>No virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>U113</td>
<td>8,506</td>
<td>12,984</td>
</tr>
<tr>
<td>U54</td>
<td>8,480</td>
<td>8,111</td>
</tr>
</tbody>
</table>

* U113 and U54 cells (T-cell hybridoma lines) were stimulated with the reovirus serotypes (10<sup>5</sup> PFU/ml) indicated. The culture supernatants were then assayed for IL-2. The results were expressed as the geometric mean. The standard deviation was within 10% of the mean. TA-3 cells (antigen-presenting B-cell hybridomas) were used after irradiation (8,000 rad).

* Culture media containing the virus were transferred to the IL-2-dependent T-cell line (HT-2), and the proliferation of this cell line was measured.
TABLE 2. Response pattern of an immunized T-cell hybridoma to live and UV-treated reovirus serotypes

<table>
<thead>
<tr>
<th>Hybridoma*</th>
<th>Presence of TA-3 cells*</th>
<th>Proliferative response (cpm) against¹:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV-treated type 1</td>
<td>Live type 1</td>
</tr>
<tr>
<td>U37T</td>
<td>+</td>
<td>11,001</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>3,839</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td>4,168</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>5,095</td>
</tr>
</tbody>
</table>

¹ U37T cells (a T-cell hybridoma line) were stimulated with the reovirus serotypes (10⁷ PFU/ml) indicated. The culture supernatants were then assayed for IL-2. The results were expressed as the geometric mean. The standard deviation was within 10% of the mean.

² TA-3 cells (antigen-presenting B-cell hybridomas) were used after irradiation (8,000 rad).

³ Culture media containing the virus were transferred to the IL-2-dependent T-cell line (HT-2), and proliferation of this cell line was measured.

U113 hybrid, seemed to respond to common determinants between live reovirus type 1 and type 3. T-cell hybridomas in group 2, such as U54 in contrast, produced IL-2 regardless of the added antigen. All T-cell hybridomas in group 1, upon restimulation with reovirus 3, gradually changed to reveal the same reactivity pattern of T-cell hybridomas as in group 2. These findings suggest that the hybridomas were induced to spontaneously produce IL-2 or developed responsiveness to some other antigens in the system.

We analyzed all of the hybridomas for infectious virus by using plaque assays of hybridoma cell lysates on L cells. All IL-2-producing hybridomas were observed to become persistently infected after restimulation with live reovirus 3. Viral proteins were also detected on the membranes of the hybridomas, as determined by flow microfluorometry (unpublished results). T-cell hybridomas such as U113 were not initially infected with reovirus 3 but became persistently infected after restimulation with live reovirus 3.

To determine the cellular requirements for IL-2 production, we evaluated the response of a persistently infected hybridoma to UV-treated and live virus in the presence and absence of irradiated TA-3 cells which we used as a source of antigen-presenting cells. Only the combination of persistently infected T-cell hybridoma cells and TA-3 cells resulted in the production of IL-2. (Table 2). We concluded that reovirus 3-specific T-cell hybridomas persistently infected with type 3 reovirus become activated upon coculture of TA-3 cells without the need for further exogenous antigen.

Establishment of persistently infected T-cell hybridomas and their specificity in activation by virus infection. To determine the reason why persistently infected T-cell hybridomas produce IL-2 after coculture of antigen-presenting cells without further reovirus stimulation, we deliberately infected reovirus-specific T-cell hybridomas with live type 3 reovirus and examined their ability to produce IL-2. However, T-cell hybridomas which were uninfected were activated only by specific viral antigen (Fig. 1). Uninfected hybridomas responded to live type 3 reovirus but not to live type 1 reovirus (Fig. 1, groups 1 through 3). These hybridomas produced IL-2 regardless of exogenous virus stimulation when cultured with the TA-3 antigen-presenting B-cell hybridoma (Fig. 1, groups 4 through 6).

In other experiments (16; data not shown) we have found that irradiated (1,500 rad) BALB/c-adherent cells, which function as antigen-presenting cells, could also replace TA-3 cells in the stimulation of IL-2 secretion by reovirus-infected T-cell hybrids. Furthermore, the antigen-presenting cells must be IA syngeneic with the T-cell hybridomas to induce IL-2 secretion. Collectively, these studies suggest that persistently infected reovirus-specific T-cell hybrids secrete IL-2 only when mixed with IA* syngeneic antigen-presenting cells.

Only live type 3 reovirus could infect reovirus type 3-specific T-cell hybridomas to produce IL-2 (Fig. 2). Other viruses, such as type 1 reovirus or UV-inactivated type 3 reovirus, were not adequate for initiating IL-2 production.

![FIG. 1. Immune responses of T-cell hybridomas after persistent infection by reovirus type 3. Two T-cell hybridomas, 997T.140 and 12T.103, had been infected with reovirus type 3 for 10 days. These hybridomas were cultured with 10⁴ TA-3 cells with or without 10⁶ PFU of reovirus for 24 h. IL-2 release was determined by using the HT-2 cell line, as described in Materials and Methods. APC, Antigen-presenting cells.](http://jvi.asm.org/)

![FIG. 2. Virus-specific activation of T-cell hybridomas by persistent infection of type 3 reovirus. The 12T.103 T-cell hybridomas had been cultured with type 1 (T1), UV-inactivated type 3 (T3uv), or live type 3 (T3) reovirus for 10 days. Infected T-cell hybridoma cells were then cultured with 10⁴ TA-3 cells without any further virus addition. Uninfected T-cell hybridomas were cultured with TA-3 cells either with or without virus. IL-2 release was determined by using the HT-2 cell line, as described in Materials and Methods. APC, Antigen-presenting cells.](http://jvi.asm.org/)
TABLE 3. Specific activation of T-cell hybridoma by persistent infection of type 3 reovirus

<table>
<thead>
<tr>
<th>T-cell hybridoma&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antigen added&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Presence of antigen-presenting cells</th>
<th>Proliferative response (cpm)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A1 (KLH specific)</td>
<td>KLH</td>
<td>+</td>
<td>15,823 ± 816</td>
</tr>
<tr>
<td></td>
<td>KLH</td>
<td>+</td>
<td>3,440 ± 756</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>2,015 ± 201</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>2,088 ± 196</td>
</tr>
<tr>
<td>A2.2B2 (HEL specific)</td>
<td>HEL</td>
<td>+</td>
<td>19,494 ± 1,940</td>
</tr>
<tr>
<td></td>
<td>HEL</td>
<td>+</td>
<td>3,494 ± 174</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>2,015 ± 201</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>2,252 ± 247</td>
</tr>
<tr>
<td>12T.103 (Reovirus specific)</td>
<td></td>
<td>+</td>
<td>14,488 ± 1,289</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>4,606 ± 1,105</td>
</tr>
<tr>
<td>Reovirus type 3</td>
<td></td>
<td>+</td>
<td>15,575 ± 934</td>
</tr>
<tr>
<td>Reovirus type 3</td>
<td></td>
<td>−</td>
<td>3,697 ± 147</td>
</tr>
</tbody>
</table>

<sup>a</sup> T-cell hybridomas (except those treated with type 3 as antigen) had been infected with reovirus for 10 days and were used as described in Materials and Methods. The hybridomas were found to contain reovirus by a standard virus-plaque assay. The titers (PFU per milliliter) were 1.0 × 10<sup>5</sup> (2A1), 7.1 × 10<sup>5</sup> (A2.2B2), and 5.0 × 10<sup>6</sup> (12T.103).

<sup>b</sup> Antigen concentrations: KLH, 500 μg/ml; HEL, 100 μg/ml; reovirus type 3, 3 × 10<sup>9</sup> PFU/ml.

<sup>c</sup> After 24 h of culture, the supernatants were assayed on the HT-2 cell line. Results are expressed as the mean plus or minus the standard deviation of triplicate cultures.

Reovirus, failed to induce IL-2 secretion. We also examined the persistently infected T-cell hybridomas (12T.103 and 99T.140) histologically but could not find any gross morphological alterations when we compared them with uninfected T-cell hybridomas (data not shown).

Moreover, to exclude the possibility that cells persistently infected with type 3 reovirus contain nonspecifically activated IL-2 genes, T-cell hybridomas with other antigenic specificities were deliberately infected with type 3 reovirus and thereafter examined for their ability to produce IL-2. Two T-cell hybridomas specific for KLH and HEL (2A1 and A2.2B2, respectively) responded to their specific antigens and produced IL-2 (Table 3). These T-cell hybrids, despite becoming infected with reovirus 3, required their nominal antigen and TA-3 cells to be induced to secrete IL-2.

Therefore, the production of IL-2 by these hybridomas was neither augmented nor inhibited by virus infection. This experiment demonstrates that persistent infection with the mammalian reovirus does not alter T-cell hybridoma specificity and does not activate IL-2 genes nonspecifically.

Furthermore, only T-cell hybridomas with reovirus 3 hemagglutinin specificity upon becoming persistently infected with reovirus 3 are able to secrete IL-2 when exposed to syngeneic antigen-presenting cells without further exogenous antigen. Collectively, these data suggest that persistently infected T cells specific for reovirus 3 only require IA<sup>+</sup> antigen-presenting cells to be induced to secrete IL-2.

The role of antigen-presenting cells in persistent infection by reovirus. We have previously shown that immunological interactions between reovirus-specific T-cell hybrids and antigen-presenting cells are IA restricted (Hinshaw et al., submitted). We infected TA-3 cells with live type 1 or type 3 reovirus to examine their influence on noninfected reovirus-specific T-cell hybrids. Only type 3 reovirus-infected or -pulsed TA-3 cells could activate reovirus 3-specific T-cell hybridomas, while type 1-infected or -pulsed TA-3 cells failed to do so (Table 4). This activation was IA<sup>+</sup> restricted (Table 5).

To evaluate the uptake, processing, and presentation of reovirus in persistently infected antigen-presenting cells, TA-3 cells were treated as described by Ziegler et al. (5, 8). Membranes of antigen-presenting cells were fixed with 0.5% paraformaldehyde to block the uptake and subsequent catabolism of virus. We also used 0.1 mM chloroquine to disrupt normal lysosomal function. Chloroquine is thought to increase lysosomal pH, leading to a subsequent depression of activity of acid hydrolases within endosomes. To inhibit reovirus receptor cycling, B-cell hybridomas were fixed with 0.5% paraformaldehyde after chloroquine treatment (5). Persistently infected TA-3 cells successfully activated uninfected T-cell hybridomas after membrane fixation by paraformaldehyde, but not after the blocking of both the intracellular processing and subsequent presentation steps by the combination of chloroquine and paraformaldehyde (Fig. 3).

We also examined whether viral presentation by uninfected TA-3 cells could be modulated by these procedures. Uninfected antigen TA-3 cells failed to present the virus to activate reovirus-specific helper T cells after being blocked either at the antigen uptake level by paraformaldehyde or at the antigen processing and presentation steps by the combination of chloroquine and paraformaldehyde (Fig. 4).

TABLE 4. Reovirus-infected TA-3 cells activate reovirus 3-specific T-cell hybrids

<table>
<thead>
<tr>
<th>Group</th>
<th>Infection of TA-3 cells&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Culture with 12T.103 cells&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Proliferative response (cpm)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Type 1</td>
<td>+</td>
<td>5,263 ± 473</td>
</tr>
<tr>
<td>II</td>
<td>Type 1</td>
<td>+</td>
<td>15,076 ± 1,301</td>
</tr>
<tr>
<td>III</td>
<td>Type 1</td>
<td>+</td>
<td>4,522 ± 226</td>
</tr>
<tr>
<td>IV</td>
<td>Type 1</td>
<td>+</td>
<td>4,080 ± 122</td>
</tr>
<tr>
<td>V</td>
<td>Type 1</td>
<td>+</td>
<td>4,302 ± 172</td>
</tr>
<tr>
<td>VI</td>
<td>Type 3</td>
<td>+</td>
<td>15,882 ± 1,317</td>
</tr>
<tr>
<td>VII</td>
<td>Type 3</td>
<td>+</td>
<td>5,296 ± 370</td>
</tr>
<tr>
<td>VII&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>+</td>
<td>5,023 ± 401</td>
</tr>
</tbody>
</table>

<sup>a</sup> In groups III, V, VI, and VII, TA-3 cells were cultured with reovirus type 1 or 3 for 10 days to induce infection. These cells were then cocultured with T-cell hybrids for 24 h without further virus addition.

<sup>b</sup> Uninfected TA-3 cells were cultured with (+) or without (−) infected 12T.103 cells in addition to type 1 or 3 reovirus groups II and IV.

<sup>c</sup> After 24 h culture supernatants were assayed on HT-2 cells. Results are expressed as the mean plus or minus the standard deviation of triplicate cultures.

<sup>d</sup> Control group (no TA-3 cells were used).

TABLE 5. Anti-IA<sup>a</sup> antibodies block the activation of reovirus-specific T-cell hybridomas by persistently infected TA-3 cells<sup>a</sup>

<table>
<thead>
<tr>
<th>Group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antibody used</th>
<th>[H]&lt;sup&gt;a&lt;/sup&gt;Bythidine incorporation (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Anti-IA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5,767 ± 567</td>
</tr>
<tr>
<td>II</td>
<td>Anti-IA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13,572 ± 1,301</td>
</tr>
<tr>
<td>III</td>
<td>Anti-IA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7,234 ± 72</td>
</tr>
<tr>
<td>IV</td>
<td>Anti-IA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13,572 ± 1,351</td>
</tr>
</tbody>
</table>

<sup>a</sup> TA-3 cells in groups II, III, and IV were infected with reovirus type 3 as described in footnote a of Table 4. MKD6 (anti-IA<sup>a</sup>) or 10-3-6 (anti-IA<sup>a</sup>) were used as blocking antibodies at a predetermined optimal concentration (50 µg/ml). Tritiated thymidine (mean plus or minus the standard deviation) incorporation was determined on HT-2 cells by using the supernatant of a 24-h culture of T-cell hybrids (12T.103) with persistently infected TA-3 cells. Group I, control (no infected TA-3 cells were used).

<sup>a</sup> All groups contain 10<sup>5</sup> 12T.103 cells.
These results indicate that there are dramatic differences in the mechanism of antigen presentation in TA-3 cells that have become persistently infected with mammalian reovirus as compared with that in uninfected TA-3 cells.

**DISCUSSION**

We have demonstrated that immunization with reovirus type 3 leads to the generation of reovirus 3-specific helper T cells. We have used these cells to create reovirus 3-specific T-cell hybridomas. The reovirus type 3-specific T-cell hybridomas were found to become persistently infected upon exposure to replicating virus. The reoviruses can be directly visualized in the cytoplasm by immunofluorescence microscopy and on the membrane by fluorescence-activated cell sorter analysis of persistently infected cells (unpublished data); the virus can be recovered, and titers can be determined in conventional plaque assays. However, we observed that persistently infected T-cell hybridomas can be

FIG. 3. The blocking of virus presentation by infected TA-3 cells to the 12T.103 T-cell hybridoma. The TA-3 B-cell hybridoma had been infected for 10 days and was treated in different ways as indicated before the coculture with T-cell hybridoma cells. After 24 h, the culture supernatants were assayed on the HT-2 cell line as described in Materials and Methods. ---, IL-2 release of 12T.103 cells in the absence of TA-3 cells.

FIG. 4. Mechanism of virus presentation to T-cell hybridomas by TA-3 cells. The TA-3 B-cell hybridomas had been treated with paraformaldehyde or chloroquine as described in Materials and Methods. Mixtures of treated B-cell hybridomas and T-cell hybridomas were cultured with reovirus type 1 (T1), type 3 (T3), or UV-inactivated type 3 T3uv for 24 h. The culture supernatants were assayed on the HT-2 cell line, as described in Materials and Methods.
induced to secrete IL-2 when mixed with syngeneic I-A\(^+\) antigen-presenting cells without the need for exogenous viral antigens.

Previous studies have identified a receptor for the mammalian reovirus type 3 on lymphoid cells. Weiner and colleagues demonstrated the interaction of the hemagglutinin of reovirus type 3 with the cell membrane of nonimmune murine and human lymphocytes (13, 44). Co et al. have successfully isolated and biochemically characterized this receptor (9, 10, 26). The receptor is a single polypeptide chain, with a mass of 65 kilodaltons and a pI of 5.9 to 6.1, which binds the hemagglutinin of reovirus type 3. In further studies, the somatic receptor was identified as structurally similar to the mammalian beta-adrenergic receptor which is expressed in high density on lymphocyte and neuronal cell membranes.

We have recently shown that two structurally distinct receptors for reovirus (hemagglutinin) recognition, namely the heterodimeric immune T-cell receptor and the mono- somic receptor (28) can be identified by a monoclo- nal anti-receptor antibody (14, 37, 38). This finding suggested that there is a configurational relationship between these two receptor systems. The mechanism by which reovirus enters cells such as the TA-3 line might occur by binding to somatic receptors on the cell surface or, alternatively, by an active phagocytic mechanism that permits viral entry without requiring specific binding to receptors. However, it is possible that reovirus might enter the T-cell hybridomas via immune receptors or somatic receptors.

Functional analyses of persistently infected lymphoid cells has been limited (23). Ahmed and Oldstone (4) have found that genetic variants of LCMV inhibit LCMV-specific cytolytic activity responses in an incompletely defined manner during persistent infection. These studies indicate that LCMV variants may demonstrate tropism for certain subsets of cells resulting in their inhibition or inactivation. Our results indicate that the helper T-cell hybrids persistently infected with reovirus type 3, rather than being functionally inhibited, can be induced to secrete IL-2 growth factors when placed in proximity to antigen-presenting cells.

Prior studies in our laboratories and others have shown that reovirus can cause persistent infections in cell culture systems (1–3). In persistent infection of mouse L cells by reoviruses, it is clear that virus undergoes extensive mutations and the cells are also affected by viral infection (1, 3). We have not studied the functional changes seen in such cells, although it is clear that in L cells there may be profound structural changes (1; unpublished data). The differences between these two viral systems (LCMV and reovirus) most likely relate to the distinct biological properties of the viruses.

Human T-cell leukemia-lymphoma virus (type I) (HTLV-1) has been shown to induce a variety of alterations in persistently infected rodent and human T lymphocytes (41, 42). Yodoi et al. found that HTLV-I infection induces the expression of IL-2 receptor proteins in infected human and rat T-cell lines (41, 45). An antigen-specific T-cell clone was observed to proliferate and secrete interferon proteins and B-cell helper factors continuously after HTLV-I infection. T cells which have been completely transformed by HTLV-I produced no detectable IL-2 in response to the specific antigen, suggesting a loss of some normal function of these cells (31, 42).

In the present study, we have observed that persistently infected reovirus T-cell hybridoma cells do not express IL-2 receptors on their cell surface either before or after reovirus exposure (unpublished observation). In addition, persistently infected T-cell hybridomas produced IL-2 only after coculture with syngeneic or semisynthetic antigen-presenting cells. These results indicate that normal major histocompatibility complex-restricted antigen recognition mechanisms remain intact in T cells persistently infected with reovirus. We are presently evaluating both reovirus-infected T-cell hybridomas and lines for the production of other growth factors.

Autoimmune IA-specific T-cell hybridomas have been previously reported by Glimcher and Shevach (18) and also by Rock and Benacerraf (34). Endres et al. (12) have developed autoimmune T-cell hybridomas specific for class I (H-2D) structures. Although the biological significance of autoimmune T-cell hybridomas is obscure, a feature noted of all the T-cell hybridomas described, including those defined herein, is their ability to produce IL-2 only when cocultured with syngeneic antigen-presenting cells. One consideration for all of these previously published results is that the putative autoimmune T cell recognizes some contaminating endogenous or exogenous virus carried in the T-cell hybridomas or the cells from which they were derived. It might be speculated that viral products are released, processed, and then presented by the antigen-presenting cells. These viral products may then stimulate the T cells in the manner described herein.

After contacting syngeneic antigen-presenting cells, persistently infected T-cell hybrids become stimulated to secrete IL-2. IL-2 is a lymphokine known to promote cell growth after interaction with IL-2 receptors present on activated T cells (24, 33). Recently Zubler et al. (49) have shown that IL-2 can also act on lipopolysaccharide and antimuramylpeptide-activated IL-2 receptor-bearing B cells. Therefore, IL-2 may act as a stimulus for B-cell and T-cell growth. T cells from autoimmune MRL-lpr/lpr mice have been found to produce IL-2 without any discernible exogenous stimulation, implicating a role of such growth factor-producing cells in the development of autoimmunity (35).

Our findings may therefore have relevance to autoimmune processes. Reoviruses are known to induce diseases including autoimmune polyendocrinopathies (29, 30). It is conceivable that cells which become persistently infected with reovirus (as do the T-cell hybridomas or antigen-presenting cells described herein) constitute components of a pathway involved in the induction and establishment of virus-related autoimmunity.

In contrast to cytolytic T cells specific for reovirus, helper T cells cannot interact with virus directly attached to the antigen-presenting cell membrane. Our results showed that these determinants had to be taken up into the cytoplasm and processed in the lysosomes to be presented in an immunogenic form after the interaction with la molecules present on antigen-presenting cells. The concept of a processing step during H-2-restricted antigen presentation has been previously proposed (46, 47). Our experimental results are consistent with these studies and suggest that the reovirus had to be internalized into an acidic intracellular compartment, after which an immunogenic moiety appeared on the antigen-presenting cell membrane. We also noted differences in the manner by which paraformaldehyde could alter persistently infected or uninfected antigen-presenting cells. Although paraformaldehyde alone limited uninfected antigen-presenting cells, it did not prevent persistently infected TA-3 cells from activating uninfected T-cell hybridomas. We have demonstrated reovirus hemagglutinin deter-
ACKNOWLEDGMENTS

These studies were supported by Public Health Service grant NS-16998-05 (to M.I.G. and B.N.F.) from the National Institutes of Health. V.S.H. is a senior scholar (grant SG-135) for the American Cancer Society and is also supported in part by the St. Jude’s Children’s Research Hospital.

LITERATURE CITED

33. Robb, R. J., A. Munck, and K. A. Smith. 1981. T cell growth factor receptors. Quantitation, specificity, and biologic rele-

Downloaded from http://jvi.asm.org/ on November 6, 2017 by guest