Detection of Mutant-specific Responses by Macrophage Migration Inhibition Reactions Induced by Wild-type and Mutant Polyoma Viruses and Polyoma Virus-infected Cells

Robert Szigeti, Tina Dalianis, Torbjörn Ramqvist, Yoshiaki Ito, and George Klein

ABSTRACT

Macrophage migration inhibition reactions of mice immunized with mutant polyoma viruses or with cells transformed and/or infected by them have been studied. The macrophage migration inhibition reaction revealed individual differences. In several cases, mice immunized with a mutant virus responded preferentially or exclusively to extracts of cells transformed or infected with the corresponding mutant. Moreover, in the macrophage migration inhibition test, mutant viruses were usually less immunogenic than were the corresponding transformed-infected cells.

INTRODUCTION

PV₃-infected normal cells have a virtually watertight immune surveillance that protects them against the outgrowth of PV-transformed cells. Their defense is T-cell dependent, but the effectors have not been clearly identified (for review, see Ref. 1). Since only the early region of the viral genome is active in transformed cells (13), the target of the host response, operationally defined as the TSTA (19), must be a direct or indirect product of the early region. It may be expected to correspond to some of the 3 known T-antigens or to a thus far unidentified, virus-induced cellular membrane component.

Recently, we have applied the MMI test (3) to the study of cell-mediated immunity in the PV system. Virus-specific responses could be obtained readily in mice immunized with wild-type PV. The nature of the antigen responsible for the MMI reaction has not been defined (21).

We have shown previously that a variety of deletion (dl) and host range (hr-t) mutants can induce TSTA-type immunity against wild-type PV-induced tumor cells (5). In the present study, we have immunized mice with the same virus mutants or with cells transformed or superinfected by them. Unlike the cross-reactive in vivo rejection (TSTA) test, the MMI reaction revealed individual differences. In several cases, mice immunized with a mutant virus responded preferentially or exclusively to extracts of cells transformed or infected with the corresponding mutant. Moreover, in the MMI test, mutant viruses were usually less immunogenic than were the corresponding transformed cells.

MATERIALS AND METHODS

Virus. The wild-type PV was a plaque-purified derivative of the A-2 Pasadena large-plaque strain (6, 9) and the parent of the mutants dl 8, dl 23, dl 1013, and dl 1015 (10, 11, 15). Mutant NG 18 has been described previously (2, 7, 12). The positions and sizes of the mutations are illustrated in Chart 1.

Cells. The cell lines used for immunization and for preparation of the antigenic extracts are listed in Table 1. The SEBA and wt CBA cell lines have been described previously (21) and contain complete S-T, M-T, and L-T. dl 8 CBA and dl 1013 CBA cells both have a small deletion in the DNA region which codes for both middle and large T-antigen (Chart 1). Therefore, these cell lines have a slightly shortened M-T and L-T. NG 18 CBA has a large deletion in DNA region common for both S-T and M-T (Chart 1). It expresses only a functional L-T. The deletion does not permit continued coding of either M-T or S-T (12).

Immunization Procedure. Groups of 8- to 12-week-old mice were immunized weekly for 5 weeks. Immunization with virus was performed as described previously (5, 21), i.e., with a virus dose of 6 hemagglutination units per mouse and week. When cells were used for immunization, they were irradiated with 10,000 R, and 10⁵ cells were inoculated s.c. per mouse and week. Both immunization schemes were sufficient for inducing the rejection of a small inoculum of living polyoma tumor cells (5).

Cell Extracts. Cells were cultured in F-13 medium with 10% NCS and harvested weekly. Crude extracts were prepared as previously (21). Washed and sonicated cells were centrifuged at 100,000 x g for 30 min at 4°C. The protein concentration of the resulting cell-free supernatant was determined (4), and the extracts were stored at -20°C until use. In each sample, 25 μg per ml protein of cell extract were used. In our previous polyoma study, this antigen concentration was sufficient to elicit a significant MMI response (21).

MMI Assay. The direct agarose microdroplet assay was performed as described before (21). Results were expressed as Ml. Ml was calculated using the formula

\[ Ml = \frac{\text{Mean area of triplicates with cell extracts}}{\text{Mean area of triplicates without cell extracts (control)}} \]

As in our earlier studies, we considered a Ml below 0.8 as a significant MMI response. For group statistics, Student's t test was used.

RESULTS

Charts 2 to 5 show the results of the MMI test following immunization with wild-type dl 8, dl 23, dl 1013, dl 1015, and NG 18 virus or with the corresponding transformed-infected cells in that order.

As a negative control extract, we have used the AKR leukemia-derived line, TIKAUT extract. As previously, it did not induce any MMI in any of the immunized mice (Charts 2 to 5).

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3 The abbreviations used are: PV, polyoma virus; MMI, macrophage migration inhibition; TSTA, tumor-specific transplantation antigen; dl, deletion mutant; hr-t, host range mutant; L-T, large T-antigen; M-T, middle T-antigen; S-T, small T-antigen; PEC, peritoneal exudate cells; Ml, migration index.

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Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Designation</th>
<th>Strain of origin</th>
<th>Induced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor of nonpolyoma origin</td>
<td>TIKAUT</td>
<td>AKR</td>
<td>Spontaneous lymphoma</td>
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<td>Polyoma virus-induced tumors</td>
<td>SEBA-TC</td>
<td>CBA</td>
<td>(A-2) polyoma virus</td>
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<tr>
<td></td>
<td>CBA</td>
<td>CBA</td>
<td>Embryoblasts transformed in vitro by (A-2) wild-type polyoma</td>
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<td></td>
<td>dl 8 CBA</td>
<td>CBA</td>
<td>Embryoblasts transformed in vitro by the dl 8 mutant virus</td>
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<tr>
<td></td>
<td>dl 1013 CBA</td>
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</tr>
<tr>
<td></td>
<td>NG 18 CBA</td>
<td>CBA</td>
<td>Embryoblasts infected in vitro by NG 18 mutant virus</td>
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* Truncated forms of L-T.

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Chart 1. PV genome early region. Map positions of virus mutants are demonstrated. Splicing of RNA is required (6, 20) for the expression of the 3 T-antigens. The cross-labeling indicates that the COOH-terminal sequences of L-T and M-T are translated in different reading frames (13). The sequences deleted by the mutants NG 18, dl 8, dl 23, dl 1013, and dl 1015 are indicated.

Chart 2. MMI in mice immunized with wt CBA cells (•). Points, means of MI of 8 experiments; bars, S.E. (*) = −− (MI, 0.8), borderline between negative and positive responses (see "Materials and Methods"). Cell extracts shown on the abscissa were applied at 25 μg/ml protein concentration.

Immunization with the in vitro-transformed wt CBA cells resulted in significant MMI when effector cells were confronted with extracts from the wild-type virus induced in vivo tumor, SEBA and wt CBA, whereas no or marginal effects were obtained with extracts of cell lines transformed or infected by the different virus mutants.

Compared to immunization with wt CBA cells and our previous findings (21), immunization with wt virus resulted in weaker MMI responses when the effector cells of immunized mice were confronted with SEBA and wt CBA extracts. Nevertheless, the difference between controls and immunized mice was significant (p < 0.05 and p < 0.02 for wt virus and wt CBA-immunized mice, respectively). Furthermore, immunization with wt CBA cells resulted in significantly higher MMI reaction than that with wt virus (p < 0.05). PEC from mice immunized with wt virus, confronted with extracts derived from cells infected and/or transformed by mutant viruses, did not show significant MMI (Chart 2).

When effector cells from mice immunized with dl 8 CBA were exposed to various antigenic extracts, only dl 8 CBA and dl 1013 CBA extracts elicited significant MMI. Immunization with the virus mutant dl 8 resulted in weak MMI only when PEC encountered dl 8 CBA extract, whereas other extracts were ineffective. Mice immunized with dl 23 mutant virus showed significant MMI only in the presence of wt CBA extract, while...
SEBA cell extract elicited only a marginal effect on the PEC of these mice (Chart 3). The mutant viruses dl 1013 and dl 1015 and CBA embryoblasts transformed by dl 1013 sensitized the effector cells of immunized mice to respond with significant MMI to the extract of cells transformed by the same mutant (dl 1013 CBA) and by dl 8 CBA. Immunization with dl 1013 virus was less effective than that with dl 1013 CBA cells (Chart 4).

NG 18 CBA cells sensitized only against themselves, but not against wt CBA, dl 8 CBA, or dl 1013 CBA extracts. NG 18 virus immunization had a similar although weaker effect (Chart 5).

DISCUSSION

Immunization with PV or with PV-transformed tumor cells induces a specific state of immunity. It is manifested by the rejection of small numbers of cells from PV-induced tumors but not from other tumors (19). Since the phenomenon was originally discovered in tumor transplantation tests, the target structure on the surface of PV-transformed cells responsible for this rejectability was termed "tumor-specific transplantation antigen." Twenty-two years after its discovery and at a time when the viral genome and its functions are known in great detail (8, 14), the nature of TSTA is as obscure as before.

It has been speculated that TSTA may represent a membrane-associated, perhaps modified form of one of the 3T antigens. While there is some evidence for such an explanation in the SV40 system, thus far there has been no compelling evidence for polyoma. The 2 systems are so different with regard to transformation mechanism and also in their immunological host-tumor relationship that any direct extrapolation is impossible (8).

In an earlier study (5), we have used a variety of deletion (dl) and host range (hr-t) mutants of PV to approach the question whether the integrity of full-length L-T or M-T was essential for the induction of TSTA immunity in rejection tests. This was not the case. Both mutants with simultaneous deletions in L-T and M-T regions and those expressing only full-length L-T with rudimentary parts of M-T were capable of immunizing mice against tumor cells induced by wild-type PV. This left us 2 possible explanations. According to the first alternative, integrity of L-T with rudimentary M-T and S-T was sufficient to induce TSTA. The difficulty with this explanation lies in the fact that L-T is not known to be associated with cell membrane at all, and even a full-length M-T is associated only with the inner surface of the plasma membrane (for review, see Ref. 8). Truncated M-T in the hr-t mutant used (NG 18) has no membrane association at all (18). According to the second alternative, some other region of the viral genome, not affected by the mutations studied, serves as the major determinant for TSTA, directly or indirectly. An indirect mechanism would involve the modification of a preexisting membrane protein or the induction of a new membrane protein, coded for by the cell.

In a subsequent study, we have applied the MMI technique to demonstrate a state of cell-mediated immunity to polyoma tumor antigens (21). This test has been used previously to study specific cell-mediated immunity to various antigens in the Epstein-Barr virus system by our group (22-24) and is a well-established method for detecting the release of migration-inhibitory lymphokines (for review, see Ref. 17) upon the confrontation of specifically sensitized effector cells with the corresponding antigen.

Following immunization with wild-type PV, we have shown that the specific MMI reaction can be obtained when the effector cells of the immunized mice are confronted with extracts from PV-induced tumors but not from other tumors of different etiology. We have not made any attempt to dissect the antigen(s) responsible for this reaction; they could be in the T-antigen category and/or TSTA.

In the present work, we have used for immunization 5 different viral mutants (1 of the hr-t and 4 of dl category) and cells transformed-infected by 3 of them, in comparison with wild-type virus and wild-type virus-transformed cells. We assessed the specificity of the MMI reaction following confrontation of the effector cells from the immunized animals with the extracts of wild-type- or mutant virus-infected-transformed cells.

The results of these experiments have been most unexpected. Four different categories could be distinguished characterized by the wild-type, dl 8/dl 23, dl 1013/dl 1015, and NG 18 virus strains and the corresponding transformed-infected cells, respectively. Within each of the categories, mice immunized with the virus or the virally transformed-infected cells responded best to the extracts of cells transformed and/or infected by the corresponding virus and failed to respond, or responded only marginally, to cells transformed and/or infected by virus in any of the 3 other groups. In each of the 4 groups, immunization with virally transformed-infected cells was more efficient than immunization with the corresponding virus. It is noteworthy, however, that a certain cross-reactivity was found between dl 8 and dl 1013 (Charts 3 and 4), although the deletions involved in these 2 mutants are completely different inside the DNA region that codes M-T (Chart 1).

Most surprising was the inability of the mutant virus (or corresponding cell)-immunized mice to respond to the extracts of cells transformed by wild-type virus. As far as viral DNA sequences and known T-antigen products are concerned, cells transformed by wild-type virus contain all information present in the mutant virus-transformed cells, except the latter have specific defects. It is noteworthy that all these defects affect the synthesis of the M-T antigen. The hr-t mutant (NG 18) affects a region to the left of the major splice site, whereas the dl mutations affect various regions to the right of it (see Chart 1). It has been shown that each of the M-T mutants produced their abnormal protein product instead of normal M-T (10, 15, 16, 18). If this is correct, each of the products, alone or following association with the cell membrane, could act as the main immunogen in the MMI test. Alternatively, if the M-T message would be instrumental in, e.g.,
triggering the cellular change that leads to TSTA-type antigenicity, a cell-coded component rather than the altered form of M-T as such would be responsible for the viral mutation-associated distinctness of the MMI.

L-T is intact in the hr-t mutants but is defective and truncated in the dl mutants. Since NG 18 virus or cells infected by it are also distinct in their antigenicity and fail to cross-react with the wild type, it is likely that differences in M-T rather than in L-T are responsible for our present finding. For some reason, these differences would particularly influence the MMI reaction, whereas the cross-reactive, possibly L-T-mediated features of the wild-type viruses and the different mutants would dominate in the rejection response. This could also be a matter of relative sensitivity. The rejection response is an all or none reaction in a given mouse. If it is more sensitive than MMI, it will indicate the cross-reactive properties, whereas the less sensitive MMI reaction will detect the "non-cross-reactive top of the iceberg."

MMI reactions performed with various PV-induced purified proteins will be helpful in the further illustration of the role of M-T in this phenomenon.

REFERENCES

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