Endogenous Retroviral Envelope Antigens Recognized by B Lymphocytes during Graft-Versus-Host Reaction

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PORTIS, J.L. Endogenous Retroviral Envelope Antigens Recognized by B Lymphocytes during Graft-Versus-Host Reactin. Tohoku J. Exp. Med., 1994, 173 (1), 83-89 — We recovered anti-murine retroviral hybridomas from the spleen cells of (B6 × D2) F₁ mice in which graft-versus-host reaction (GVHR) had been induced by the infusion of spleen cells from either parent. The antibodies were specific for envelope proteins of endogenous retroviruses, and have provided a convenient way of classifying viruses based on their host range. They also have revealed considerable serologic heterogeneity within each host-range group. We have utilized these antibodies to characterize endogenous envelope glycoproteins in uninoculated mice. In this report I have attempted to synthesize the results of studies carried out by a number of senior investigators as well as postdoctoral fellows who have graced the Laboratory of Persistent Viral Diseases over the last 10 years. Some of the information on the nature of the endogenous glycoproteins expressed within the hematopoietic compartment of DBA/2 and C57BL mice has provided a basis for speculation, offered at the end of the report, on the possibility that anti-retroviral autoantibodies generated during the course of GVHR may be antigen-driven. — endogenous retrovirus; graft-versus-host reaction; B lymphocytes; monoclonal antibodies

In the last few years the importance of retroviral gene products as superantigens (Acha-Orbea and Palmer 1991; Choi et al. 1991) and their potential influence on the T cell repertoire (Pullen et al. 1990) has focussed attention on endogenous retroviral sequences of both mice and man. The mouse genome is replete with viral sequences related to type A (intracisternal particles), type B (mammary tumor virus) and type C (oncornavirus) retroviruses. We have studied the envelope proteins encoded by members of the latter group in normal uninoculated mice. In this report I will describe the generation of mouse monoclonal antibodies which recognize some of these proteins and the use of these reagents to identify cells which express these proteins.

Before describing these experiments, however, a brief overview of terminology is in order. Murine type C retroviruses have been classified into four

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host-range groups, based on their cellular tropism in vitro. Ecotropic viruses infect mouse cells only. Xenotropic viruses fail to infect mouse cells but infect cells from other species. Polytropic and amphotropic viruses infect mouse cells as well as cells from other species. The species tropism exhibited by each of these groups is a function of the viral envelope sequences, and appears to define at least four different cell surface receptors (Rein and Schultz 1984). Thus, the inability of an ecotropic virus to infect non-mouse cells can be overcome by pseudotyping the ecotropic viral RNA in the envelope protein of, for instance, a polytropic virus. Alternately, exchanging the envelope gene of an ecotropic for that of a polytropic virus yields a virus with the tropism of the polytropic parent.

Retroviruses utilize a cDNA copy of their viral RNA as a replication intermediate. This viral DNA becomes stably integrated in the cellular chromosomal DNA. When integration occurs in a germ cell, the retroviral sequences then are carried from generation to generation and segregate in a Mendelian fashion. Most of the retroviral sequences within the mouse genome appear to be transcriptionally silent. However, there are examples of expression at the protein level and rarely entire relication competent retroviruses can be expressed. In the latter case, as is seen in certain mouse strains such as AKR and C58, endogenous retroviruses are responsible for the induction of leukemia. In the current report we will focus on viral envelope proteins expressed in normal mice.

Generation of monoclonal antibodies reactive with endogenous retroviral envelope proteins

Since mice are generally tolerant to endogenous retroviral proteins, we generated the hybridomas by induction of autoimmunity, specifically graft-versus-host reaction. Parental spleen cells from either DBA/2 (D2) or C57BL/6 (B6) mice (50-100 X 10^6) were inoculated intravenously into (B6 X D2) F1 recipients. Ten to sixty days later we carried out the cell fusions with spleen cells of the recipients, and the resulting hybridoma cells were screened using a membrane immunofluorescence assay on cells which were confluently infected with different murine retroviruses (Portis et al. 1982; Portis and McAtee 1983).

Interestingly, although all of the antibodies detected by this method were directed to env proteins (gp70, p15E or gp85^{env}), the retroviral specificities of the monoclonal antibodies derived from these two parent→F1 combinations were strikingly different (Table 1). Many hybridomas derived from F1 recipients of D2 spleen cells produced antibodies reactive with xenotropic as well as ecotropic viruses, and several of the antibodies were directed at common determinants on the envelope glycoproteins of ecotropic, xenotropic and polytropic retroviruses. In contrast, many of the antibodies derived from F1 recipients of B6 spleen cells reacted specifically with the glycoprotein of polytropic viruses, being unreactive with either ecotropic or xenotropic viruses. We analyzed the allotype of several of the IgG2a monoclonal antibodies derived from F1 recipients of D2 spleen cells,
and they bore the IgIb allotypic marker from the B6 parent, indicating that they were of recipient origin. The dependence of viral specificity on the origin of the donor cells was a mystery until, with the aid of some of these antibodies, we learned more about the types of retroviral envelope glycoproteins expressed by the two parents. We will therefore return to this point later in this report.

Using these antibodies we were able to classify xenotropic retroviral env proteins into 4 serotypes (Portis et al. 1982) and polytropic viruses into 10 serotypes (Portis and McAtee 1983). Thus, these antibodies have revealed a high level of heterogeneity among endogenously derived xenotropic and polytropic viruses, and have been indispensable in their identification, quantification and localization in the tissues.

**Characterization of endogenous retroviral envelope proteins in normal mice**

Friend virus complex (a mixture of a defective virus SFFV and a replication competent helper virus F-MuLV) induces a massive polyclonal expansion of erythroid cells. (B10.A × A)F1 mice, by virtue of their possessing certain resistance genes, survive this acute proliferative response and develop true oligoclonal erythroleukemia. Cell lines derived from the leukemic spleens of these mice proliferate autonomously in vitro. Britt et al. (1984) observed that during the early proliferative phase of Friend disease, spleen cells expressed high levels of envelope glycoprotein of the F-MuLV helper virus, but during the late leukemic phase, there was a loss of this protein, associated with the simultaneous appearance of a unique xenotropic gp70 reactive with one of the GVHR antibodies 18–6. The 18–6 antibody detects a gp70 epitope which defines a subclass of xenotropic viruses (Portis et al. 1982). Erythroleukemia cell lines derived from Friend virus-infected mice stably expressed the 18–6-positive gp70 protein in vitro. Expression of this protein did not depend on infection by an exogenous retrovirus, since a protein with similar serologic reactivity and structure, based on two dimensional chymotryptic peptide maps, was also seen on midgestation fetal liver cells from these mice. This protein was also found to be expressed by primary embryo as well as dermal fibroblast cultures. Thus, the 18–6-reactive gp70 was the product of an endogenous env gene and appeared to mark the transformed cells which eventually emerged late in the course of Friend disease.

**Table 1. Viral specificities of GVHR monoclonal antibodies**

<table>
<thead>
<tr>
<th>Donor/Recip.</th>
<th>E + X + P ( ^a )</th>
<th>X + P</th>
<th>X</th>
<th>P</th>
<th>Total</th>
<th># Mice</th>
</tr>
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<tbody>
<tr>
<td>D2/(B6xD2) F1</td>
<td>4 ( ^b )</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>B6/(B6xD2) F1</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>17</td>
<td>4</td>
</tr>
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\( ^a \) E, ecotropic; \( ^b \) X, xenotropic; P, polytropic.

Number of hybridomas producing antibodies with designated viral specificities.
With the aid of other monoclonal antibodies, we went on to identify other endogenous retroviral envelope proteins. One of these proteins reacted with monoclonal antibody 514 which detects a common epitope expressed by virtually all polytropic viruses tested to date. This endogenous polytropic gp70 was detected on fetal liver cells as well as dermal fibroblasts of D2 mice, a strain which is negative for the 18–6-reactive gp70 seen in (B10.A × A)F₁ mice. On screening a number of other mouse strains for the 18–6 and 514-reactive gp70 proteins (Table 2), we found that no strain expressed both proteins and a number of strains were negative for both proteins.

Genetic experiments indicated that the 18–6-reactive gp70 of B6 mice and the 514-reactive gp70 of D2 mice behaved as alleles, as no recombinants were seen in over 100 crosses, and these alleles were expressed codominantly. These viral proteins were expressed by approximately 50% of fetal liver cells at midgestation, and the majority of these cells were of the erythroid lineage. Interestingly, in adult mice we were unable to find cells expressing these proteins within hematopoietic organs (i.e., spleen and bone marrow), although high levels of xenotropic gp70, reactive with other GVHR-derived monoclonal antibodies, were found within these tissues (Buller et al. 1989). Upon stimulation of erythropoiesis by treating adult D2 or B6 mice with the hemolytic agent phenylhydrazine, a small population of cells expressing respectively the 514 and 18–6-reactive gp70’s were detected in the spleen (Buller et al. 1989). This illustrates the power of discrimination of the monoclonal antibodies used in these studies, allowing the detection of several endogenous envelope proteins expressed within the same organ.

On analysis of the lineage distribution of the 514/18–6-reactive gp70 proteins (Buller et al. 1989) it was found that these proteins did not just mark erythroid cells, but were expressed by cells of the myeloid, granulocyte/macrophage and lymphoid lineages as well. Expression of these proteins was detected from primitive stem cells (CFU/S) through more mature progenitors such as CFU/GM, CFU/E and BFU/E. However, neither protein was detected in mature forms of

<table>
<thead>
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<th>Table 2. Mouse strain distribution of env proteins*</th>
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<tr>
<td>18–6+</td>
</tr>
<tr>
<td>C57BL/6</td>
</tr>
<tr>
<td>C57BL/10</td>
</tr>
<tr>
<td>CBA/J</td>
</tr>
<tr>
<td>A/WySn</td>
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*Primary tail skin cultures were analyzed by flow cytometry for membrane immunofluorescence with the monoclonal antibodies 18–6 and 514. Fetal liver cells and/or embryo cell cultures were also examined for expression of the respective gp70 proteins in C57BL/6, C57BL/10, DBA/2, and IRW.
any of these lineages.

Function of the 514/18-6-reactive endogenous envelope proteins

Expression of endogenous retroviral proteins within tissues of the developing fetus had long been recognized, and it was suggested that these proteins may have some function in embryogenesis. Although little support for this idea has been forthcoming, endogenous retroviruses have been shown to perform at least one function which was originally identified among wild mice. In two independent genetic studies on feral mice in Asia and the Western US, a gene was identified which was found to prevent leukemia and neurodegenerative disease caused by indigenous ecotropic retroviruses. This gene, termed *Fv-4* in Asian mice (Odaka et al. 1978; Ikeda and Odaka 1983) and *Akvr-1* in North American wild mice (Gardner et al. 1980), encodes an ecotropic retroviral envelope protein and appears to restrict spread of ecotropic viruses by a mechanism of receptor blockade, also called viral interference.

D2 mice exhibit genetic resistance to erythroleukemia induced by neonatal inoculation of F-MuLV, the helper virus of the Friend virus complex. Resistance is genetically linked to the *Rmcf* locus, first identified and mapped by Hartley and coworkers (Hartley et al. 1983). This locus appears to also mediate viral interference and restricts specifically infection by polytropic viruses. In genetic studies, we found that the *Rmcf* allele segregated with the 514-reactive gp70 of D2 mice (Buller et al. 1987), and this protein also appeared to segregate with resistance to erythroleukemia (Buller et al. 1988). Furthermore, the 18-6-reactive gp70 of C57BL mice appeared to function as a resistance gene for a brain disease caused by a neurovirulent polytropic virus (Buller et al. 1990). Expression of both the 514 and 18-6-reactive gp70 in vivo is associated with a restriction of replication and/or spread of recombinant polytropic viruses (Buller et al. 1988; Buller et al. 1990). Whether these two *env* genes are encoded by the *Rmcf* locus itself is not yet clear. In linkage studies carried out by Coffin and his colleagues (Stoye and Coffin 1988; Frankel et al. 1989) using oligonucleotides specific for endogenous xenotropic and polytropic envelope sequences, no detectable proviral sequence mapping to the *Rmcf* locus was identified. Thus, the *Rmcf* locus could represent a regulatory sequence controlling the expression of the *env* structural genes. Nevertheless, the function of endogenous retroviral sequences as resistance genes in retroviral diseases emerges as a recurring theme, and may explain the retention of some of these sequences within the mouse genome.

Concluding remarks

As mentioned at the outset, during generation of the anti-retroviral hybridomas from mice undergoing GVHR, the viral specificities of the resulting monoclonal antibodies appeared to be a function of the source of the donor spleen cells (Table 1). Thus, in the B6→(B6×D2)F1 combination, a large group of
monoclonals was recovered which reacted with polytropic virus-specific epitopes. In contrast, no polytropic-specific monoclonals were recovered from the \((B6 \times D2)\) F\(_1\) recipients of D2 spleen cells. If the hybridomas were, indeed, derived from recipient B lymphocytes, as the allotype analysis would suggest, then the skewing of specificities may be a function of T-cell tolerance induced by the endogenous envelope proteins expressed in the donor mice. This would, in turn, imply that the anti-retroviral autoantibodies induced during GVHR were antigen driven, dependent on the introduction of antigen-specific T lymphocytes of donor origin.

Further understanding of the complexity of the retrovirus-derived antigen system of mice will require the cloning and sequencing of the genes encoding these proteins. Stoye and Coffin and their collaborators have focussed their attention on the genomic sequences and have discovered a large number of proviral sequences scattered apparently randomly throughout the mouse genome, and have classified these, based on Southern blot analysis, into three classes, xenotropic, polytropic and modified polytropic (Stoye and Coffin 1988). The random distribution of these proviruses is proving very useful in the chromosomal mapping of other mouse genes (Frankel et al. 1990).

The importance of endogenous retroviruses in autoimmune diseases is unclear at this time. However, their potential for mutation and recombination, as well as their capacity to capture and move normal cellular genes, makes endogenous retroviruses a possible rich source of neoantigens which, by virtue of their expression within the tissues, may serve as targets for autoimmunity. Further analysis of sequence heterogeneity of endogenous murine retroviral envelope genes, through cDNA cloning of their transcripts, is currently under way.

Acknowledgments

This work was carried out over the last 10 years, as a collaborative effort, by a number of investigators working both in my laboratory and in the laboratory of Dr. Bruce Chesebro. I want to thank each of them for their contributions. These individuals have since moved on to other venues, but they should be proud of their accomplishments while at the Rocky Mountain Laboratories. They include Dr. William J. Britt, Dept. of Pediatrics, University of Alabama School of Medicine, Birmingham Alabama; Dr. Richard S. Buller, Dept. of Pediatrics, Washington University School of Medicine, St. Louis Missouri and Dr. Masaaki Miyazawa, Dept. of Pathology, Tohoku University School of Medicine, Sendai. I would also like to thank the organizers of “International Symposium on Genes and Molecules in Autoimmune Diseases” held in Sendai to honor the distinguished career and accomplishments of Professor Masahisa Kyogoku.

References


