Tohoku J. Exp. Med., 1994, 173, 105-114

Role of Endogenous Retroviruses in

Autoimmune Diseases

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GOURLEY, M.F., KISCH, W.J., MOJCIK, C.F., KING, L.B., KRIEG, A.M. and STEINBERG, A.D. Role of Endogenous Retroviruses in Autoimmune Diseases. Tohoku J.Exp.Med., 1994, 173 (1),105-114 Retroviruses have been implicated in the pathogenesis of murine and human lupus; however, many positive findings have been followed by alternative explanations. Initial findings implicating xenotropic retroviruses were subsequently invalidated. The first solid demonstra-tion that endogenous retroviruses mediate disease was the study of SL/Ni mice. Here budding ecotropic retroviral particles from arterial smooth muscle cells caused an antibody response to the particles with subsequent complement deposition. Our laboratory has focused on derangements in endogenous MCF retroviral expres-sion. We found that lupus-prone NZB, BXSB and MRL strains have a marked increase in expression of Mpmv RNA in their thymuses while bone marrow expression did not differ from normal strains. Sequence analysis demonstrated mutations in the NZB endogenous retroviruses which could alter expression. A phosphorothioate antisense oligonucleotide to the initiation sequence of Mpmv
caused lymphocyte activation in vivo in normal mice, providing further evidence
for in vivo effects of Mpmv and potential for pathological abnormalities in lupus-prone strains. endogenous retrovirus; systemic lupus eryth-
ematosus; autoimmunity; antisense; polytropic retrovirus

Although the causes of human and murine systemic lupus are unknown, a substantial body of evidence implicates genetic and environmental factors.

When bacterial infections could not be implicated, a search for a virus was launched. Retroviruses have long been suspected as a potential etiologic agent. Initial findings in NZB mice suggested that xenotropic viruses play an important role in disease mediation (Yoshiki et al. 1974). It was found that immune complex kidney disease resulted, at least in part, from gp70-anti-gp70 immune

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complexes (Yoshiki et al. 1974). However, further work through backcross studies dissociated xenotropic retroviruses from kidney disease (Datta et al. 1978). The first report of an endogenous ecotropic retrovirus being implicated in host tissue destruction was made by Miyazawa et. al. (1987). Using the SL/Ni mouse model for autoimmune disease, budding retroviral particles were seen coming from the arterial smooth muscle. This in turn led to a complex of antibody formation, immune complex deposition and subsequent inflammation in the vessel wall. Unfortunately, this strain did not breed well and was ultimately lost to further analysis. Nevertheless, other mice including NZB, BXSB and MRL are available for analysis of endogenous retroviral expression.

Systemic lupus: A generalized autoimmune disease
Studies of recombinant inbred mice derived from parental lupus-prone NZB and non-autoimmune mice have suggested that approximately 6 independently segregating genetic loci or "background genes" may contribute to disease (Raveche et al. 1981; Miller et al. 1984). Some of these "background genes" appear to regulate the general state of immune activation and self-tolerance. In addition, the single recessive genes, lpr and gld, are known to be responsible for the early appearance and rapid progression of disease (Smith and Steinberg 1983; Steinberg et al. 1990). Both of these genes lead to a marked increase in T cell mass and autoantibody production, even when bred onto normal strains of mice; however, the syndrome develops more rapidly and completely on an autoimmune back-ground. The autoimmunity accelerating gene present on the Y chromosome of BXSB mice apparently acts to accelerate disease on an autoimmune background but cannot induce it on a normal genetic background (Hudgins et al. 1985). These single genes (lpr/lpr, gld/gld and BXSB-Y) all lead to polyclonal B cell activation (Ishigatsubo et al. 1988; Klinman et al. 1988, 1990). The gld gene has been mapped to a region of distal chromosome 1 of the mouse (Seldin et al. 1987). The lpr gene has recently been cloned and mapped to chromosome 19 and potentially linked to a ligand receptor (Watanabe-Fukunaga et al. 1992a, b). As a result, the relationship between genetic predisposition to disease and the mechanisms of disease induction has begun.
One line of investigation by which we have approached this question of the genetic basis of lupus is through studies of endogenous retroviruses. In this report, we describe our examination of the relationship between endogenous type C retroviral expression and murine lupus.

Endogenous type C murine retroviruses
There are three major classes of murine type C retroviruses: ecotropic (which infect mouse cells), xenotropic (which infect non-mouse cells) and MCF, mink cell focus-forming (which infect both mouse and non-mouse cells and, therefore, are also called polytropic). Infectious ecotropic and xenotropic retroviruses can be

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found in the genome. In contrast, infectious MCF retroviruses are derived by recombination of several endogenous retroviral sequences. Despite the existence of a small number of infectious retroviral sequences in the genome, most en-dogenous retroviral sequences are not infectious.
It appears that at least 1 to 5% of the human and mouse genomes have arisen through reverse transcription of retroviral and non-retroviral sequences (Balti-more 1985). One class of endogenous type B retroviruses are the mouse mammary tumor viruses (MMTV) which have been shown to play an important role in the selection of specific T cell receptor usage in mice (Acha-Orbea and Palmer 1991; Acha-Orbea et al. 1991; Choi et al. 1991). These as well as other xenotropic, ecotropic and MCF loci have been chromosomally located (Frankel et al. 1990). It is believed that type C endogenous retroviruses are capable of encoding functional protein. Two well characterized env proteins include gp70 and pl5E. Recent studies suggest that gp70 is capable of transforming growth-factor depen-dent cells to become growth-factor independent (Li and Baltimore 1991; Tsichlis and Bear 1991). pl5E is a well recognized immunosuppressant molecule (Mathes et al. 1978). Altering expression of these proteins by various manipulations helps to delineate the role of endogenous retroviral expression in immune regulation.

Antisense studies

We examined possible effects of endogenous retroviral expression by use of antisense oligodeoxynucleotides. In particular, we hypothesized that if proteins encoded by endogenous retroviral sequences normally inhibited lymphocyte activation, the antisense might interfere with such inhibition and allow activation (Krieg et al. 1989; Steinberg et al. 1991). The first such studies were performed in vitro with conventional oligonucleotides (Krieg et al. 1989). Spleen cells from normal mice incubated with antisense to the MCF-env initiation region did increase RNA and DNA synthesis. Scrambled oligonucleotides failed to do this. Release from inhibition also was not observed with anti-sense to ecotropic or xenotropic env. Rat cells, which do not contain the murine MCF sequences, were not stimulated, suggesting that the effect did not result from non-specific stimula-tion (Krieg et al. 1989). The magnitude of the effect of antisense was much increased when nuclease resistant phosphorothioate antisense oligonucleotides (Steinberg et al. 1991) were used (Table 1).
These data suggest that endogenous MCF-related retroviral sequences exert
an inhibitory regulatory effect on lymphocyte activation. We believe that this represents an adaptation of mammals to the endogenous retroviral sequences which have integrated into their genomes.
This in vitro work then expanded to in vivo studies in which mice were injected with comparable phosphorothioate oligonucleotides. Increased RNA synthesis were found in mice injected with anti-sense to MCF-env as compared with mice injected with scrambled anti-sense or with anti-sense to env of

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xenotropic or ecotropic retroviral sequences. Anti-sense injected mice also had increased numbers of spleen cells, primarily activated B cells (Mojcik et al. 1993).

Full-length (8.4 kb) modified polytropic (Mpmv) thymic transcripts are characteris-tic of murine lupus
Oligonucleotide probes able to distinguish xenotropic from endogenous MCF-like type C retroviruses were used in Northern analyses of RNA. These studies confirmed that there was no association between xenotropic expression and murine lupus. However, endogenous full-length (8.4 kb) MCF transcripts were found in the thymuses of autoimmune-prone NZB, MRL-lpr/lpr, BXSB, and (NZB x NZW) F1 mice (Krieg and Steinberg 1990). These 8.4 kb MCF RNA were not highly expressed in control strains.
Subsequently, it was found that two subsets of endogenous MCF sequences, Pmv and Mpmv, could be distinguished by subclass-specific oligonucleotide probes (Krieg and Steinberg 1990). Studies with these subclass-specific probes on RNA from thymic tissue demonstrated that 8.4 kb Mpmv expression is highly correlated with murine lupus. This RNA is present at maximal levels from birth in NZB mice, strongly suggesting that these transcripts do not result from the autoimmune process.
The studies of 8.4 kb Mpmv expression may not be related directly to the functional env antisense studies presented above. Further work will be required to determine whether any such relationship exists.

Bone marrow studies

In an attempt to localize the thymic 8.4 kb Mpmv expression, subsets of thymocytes were studied. Further, greater 8.4 Mpmv expression was found in CD 4~, CD 8- thymocytes than in single positive or double positive thymocytes. This expression in the most immature, CD 4-, CD 8- thymocytes pointed to a bone marrow origin. We then analyzed whole bone marrow for Mpmv expression. The results clearly showed that all strains of mice have full-length Mpmv tran-

TABLE 1. Antisense oligonucleotides to MCF retrovirus env initiation site exhibit an inhibitory effect in lymphocytes thereby allowing
increased activationa

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scripts. Therefore, the thymuses of lupus prone mice differ from those of normal mice. The pre-T stem cells in the bone marrow carry 8.4 Mpmv expression which is subsequently lost when these cells migrate into the thymus of normal mice. Additional work with reciprocal repopulation studies were done between BXSB and C57BL/6 mice (Table 2). The bone marrow donor determined the recipient thymic 8.4 kb Mpmv expression. Similarly, T cell depleted bone marrow from NZB donors was used to repopulate lethally irradiated Balb/c and DBA/2 recipients (Krieg et al. 1991). There was high expression of 8.4 kb Mpmv RNA in the recipients 4 weeks after transfer. Reciprocal repopulation studies of heavily irradiated NZB recipients demonstrated a loss of 8.4 kb Mpmv expression. Thus, full-length Mpmv thymic expression, like the tolerance defect of ,NZB mice (Laskin et al. 1982), appears to map to the pre-T bone marrow stem cell.

Sequence analyses
To further characterize subsets of type C endogenous retroviruses, a genomic DNA library from an NZB mouse was made. Genomic DNA was isolated from liver, partially digested with Hind III and ligated into a pWE15 vector. The library was screened and a full length Pmv (876) and a Mpmv (12502) clone was isolated. The sequences of the env and 3' LTR were compared with those published by Stoye and Coffin (1987) (Fig. 1). The 876 Pmv clone contains the correct ATG initiation site and the entire env contains an open reading frame. There are a total of 19 point mutations, 14 in the gp70 portion of the molecule and 5 in pl5E. These mutations translate into 8 amino acid changes in gp70 and 3 in p15E. Interestingly, all mutations within p15E involve changing the basically charged lysine to an acidically charged glutamic acid.
Clone 12502 is fully homologous to MX33 (Mpmv) until position 1556 where

TABLE 2. Association of 8.4 kb Mpmv RNA and surface retroviral envelope
gp70 expression with autoimmune donor in radiation chimeras

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12502 has an guanosine insertion. This insertion closes the open reading frame prematurely, causing the p15E molecule to be truncated. A single base change is found in the LTR at position 2075 of cytosine instead of thymidine. This region of the LTR is a highly conserved negative regulatory region. Computer analysis of the translated protein products fails to reveal any difference in the prediction of antigenic determinants as determined by the Hopp and Woods method.

Comments

Lupus-prone strains expressed substantial amounts of thy mic 8.4 kb mRNA

Fig. 1. Sequence analysis of endogenous retroviral clones 876 and 12502. These
sequences are compared to Stoye and Coffin's prototypes MX27 and MX33
(Stoye and Coffin 1987) which appear in their entirety with only the base
changes shown for 876 and 12502. The translated product appears with the
protoypes, only changes appear with 876 and 12502.

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(full-length murine retrovirus) encoded by endogenous MCF-related retroviral sequences. The 8.4 kb Mpmv subset of MCF-related transcripts in the thymus is characteristic of murine lupus and not secondary to the immune activation which occurs in disease.

The unique expression of full-length (8.4 kb) Mpmv endogenous retroviral transcript in the thymuses of autoimmune-prone mice provides a possible clue to early gene dysregulation in murine lupus. Even if the retroviral transcripts do not induce disease, they probably result from factors which lead to pathogenic

events.

Fig. 1. (continued)

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The studies of radiation chimeras have pointed to the bone marrow pre-T
stem cell as the carrier of the abnormality in 8.4 kb Mpmv expression. Similarly, previous studies of experimental tolerance in NZB mice mapped the defect to pre-T stem cell. Moreover, we recently have found 8.4 kb Mpmv transcripts in murine marrow, from normal and lupus-prone strains of mice. Therefore, bone marrow cells carry the RNA transcripts of Mpmv in all strains of mice but strains which develop murine lupus have abnormal expression which persists in the thymus.
Retroviral gene products are able to influence the immune system. Our
antisense studies confirm these findings. If proteins such as pl5E normally
inhibit lymphocyte activation, antisense oligonucleotides which interfere with such inhibition would allow immune activation. Our studies confirm this hypothesis as antisense to the initiation of the env gene allows lymphocyte
activation as demonstrated by increased DNA and RNA synthesis and cell surface
activation markers.
The sequences of both Pmv andMpmv have been analyzed. An NZB Mpmv

Fig. 1. (continued)

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clone studied has an insertion in the p15E portion of the sequence which allows the p15E molecule to be truncated. This would lead to the loss of the intact p15E molecule and therefore the loss of its immunosuppressive effect. Furthermore, a

base change in the negative regulatory region of the LTR may result in a higher constitutive expression of the Mpmv transcript. Both of these findings could

contribute to state of chronic lymphocyte activation found in autoimmune

diseases such as systemic lupus erythematosus.

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