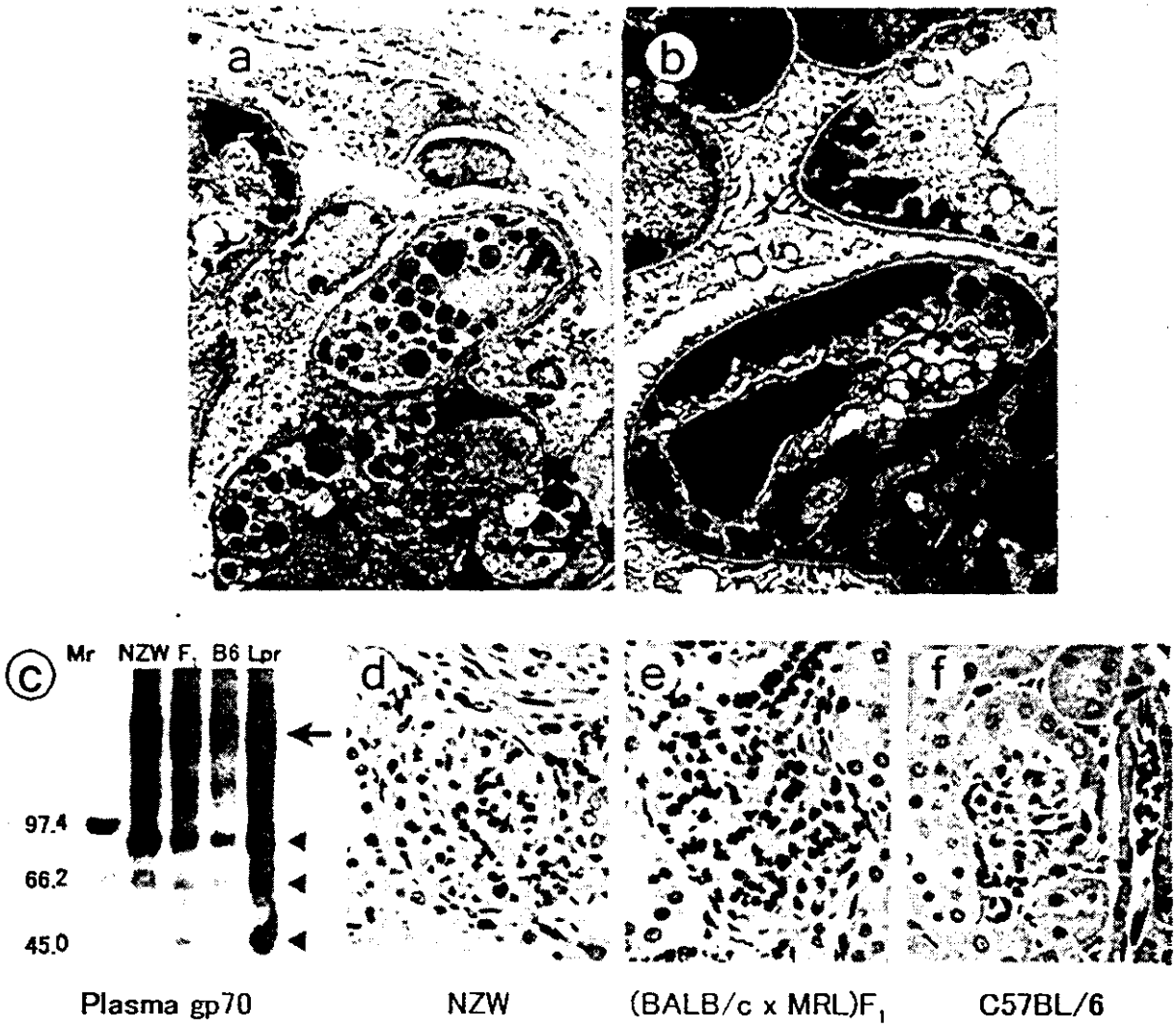

Fig. 1: Pathogenicity of monoclonal anti-gp70 autoantibodies established from MRL/lpr lupus mice shown by passive transfer into non-autoimmune mice. a, An electron micrograph showing cellular proliferation filling the glomerular capillaries and accumulation of electron-dense droplets in the cytoplasm of glomerular cells in (BALB/c x MRL)F1 mice transplanted with anti-gp70 antibody-producing hybridoma cell clone, 12H5.1. Bar = 5 mm. b, Massive deposition of electron-dense materials between the basement membrane and the endothelial cells in the glomeruli of a representative (BALB/c x MRL)F1 mouse transplanted with another anti-gp70 autoantibody-producing hybridoma cell line, 37C4.1. Bar = 2 mm. c, A representative western blot analysis showing differences in serum gp70 expression in NZW, (BALB/c x MRL)F1, and B6 mice. Sera were diluted 1:20 into the SDS-sample buffer without a reducing reagent, boiled for 5 min., and 10 ml of each boiled mixture was loaded into a well of 7.5 % polyacrylamide gel. Plasma from a 4 month-old female MRL/lpr mouse (Lpr) that should contain a large amount of gp70-anti-gp70 immune complexes, was used as a positive control. NZW mice expressed a high level of serum gp85 (gp70 + p15E), gp70, and a degradation product gp45 (arrowheads), while their expression in B6 mice was low. (BALB/c x MRL)F1 mice (F1) expressed an intermediate level of serum gp70. Mr, biotinylated markers with numbers on the left showing relative molecular mass x 10–3. The viral envelope gp70 is known to form trimers, and the corresponding band of apparent molecular mass > 220 kDa was observed (arrow). d-f, Representative photomicrographs taken from kidney sections of NZW (d), (BALB/c x MRL)F1 (e), and B6 (f) mice injected with purified anti-gp70 IgG3, 12H5.1.

Hematoxylin and eosin staining, x 300. Note apparent thickening of the capillary walls and inflammatory cell infiltration in d, and the marked increase in glomerular cellularity and evident neutrophilic infiltration in e.

Immunoglobulin variable region gene usage and amino acid sequences of the pathogenic anti-gp70 autoantibodies

Immunoglobulin heavy and light chain V gene usages and amino acid sequences of the above pathogenic anti-gp70 autoantibodies established from MRL/lpr lupus mice were analyzed by determining nucleic acid sequences of the cDNAs (Tab. 1). Four of the 5 examined clones were using the germ line VH558 family gene which is known to be preferentially used by many anti-DNA antibody clones, including those established from an MRL/lpr mouse [25,26]. VH7183 family gene that is used by the anti-gp70 clone 51D1.1 is also known to be used preferentially by anti-DNA antibodies in (NZB x NZW)F1 mice [26]. Accordingly, the heavy chain CDR1 and CDR2 amino acid sequences of the examined pathogenic anti-gp70 autoantibodies are similar to those reported for the anti-DNA antibody clones established from the MRL/lpr mouse. However, the observed amino acid sequences of heavy chain CDR3 of the pathogenic anti-gp70 antibodies were quite dissimilar to those reported for anti-DNA antibodies, suggesting that CDR3 is mainly involved in the determination of anti-gp70 specificity of these antibodies. In addition, a high frequency of Arg or Lys residues in CDR3 that is know to favor DNA binding of autoantibodies [26,27] was not a common feature of the examined anti-gp70 antibodies, and accumulation of these basic residues in CDR3 was observed in only two of the 5 examined clones. Interestingly, these 2 clones, 36D1.1 and 37C4.1, established from a single fusion, shared the identical heavy chain gene sequence and hence the CDR amino acid sequences, but differed in their light chain gene usages. These results suggest that the two clones have probably developed from a common pre-B cell. Further, since observed pathogenicities of the two clones were different, pathogenic potentials in these two clones of anti-gp70 autoantibodies might be determined by their light chain amino acid sequences. Further analyses are required to experimentally prove this possibility.

Induction of granulomatous arteritis by injection of purified anti-gp70 monoclonal antibody into non-autoimmune mice

During the process of examining glomerular pathology in anti-gp70 antibody-injected animals, we noted the presence of granulomatous arteritis in the lungs of some injected mice. The lesions were characterized by dense accumulation of neutrophyls and macrophage-like cells in the adventitia with focal destruction of the muscular media. Subendothelial accumulation of neutrophils in the intima was also observed, but less frequently. Small

arterioles were mainly involved, but larger branches of pulmonary arteries were also affected at a low frequency. To systemically analyze the development of this arterial pathology, we purified large amounts of the anti-gp70 and control anti-SRBC IgG3 antibodies, and injected them into (BALB/c x MRL)F1, (B6 x MRL)F1, and NZW mice. A total of 3-6 mg/mouse of a purified antibody was injected in 6 to 10 split doses of 0.7 ml twice a day for 3 to 5 days, and injected animals were examined 2 days after the final injection based on the results of preliminary experiments in which pathological analyses were done 1, 2, 4, and 7 days after the final injection. The granulomatous arterial lesions were observed only in mice injected with anti-gp70 clone 12H5.1, but not in those injected with 51D1.1 or control anti-SRBC IgG3, N-S.7. The frequency of the development of the arteritis was higher in NZB mice than in (BALB/c x MRL)F1, suggesting possible involvement of gp70-anti-gp70 immune complexes. More detailed analyses on the pathogenesis of this granulomatous arteritis are currently under way, and will be reported separately.

Tab. 1. Immunoglobulin variable region gene usage and deduced amino acid sequence of pathogenic anti-gp70 autoantibodies.

Clone	Isotype	V_H	J_H	$V\kappa_\kappa$	$J\kappa_\kappa$
12H5.1	IgG_3, κ	558	4	21	2
36D1.1	IgG_{2a}, κ	558	3	19-20	2
37C4.1	IgG_3, κ	558	3	8-21	4
37C6.1	IgG_{2a}, κ	558	3	10	2
51D1.1	IgG_3, κ	7183	4	21B	1

Clone	V_H		
	CDR1	CDR2	CDR3*
12H5.1	RYWMH	AIYPGNSDTSYNQKFKGK	EGISIYDGLYFAMDY
36D1.1	DYSMD	YIYPNNGYTGYNQKFKSK	KLGRREAYFDV
37C4.1	DYYMD	YIYPNNDGTNYNQKFKGK	GGLAGYYLYYAMDY
37C6.1	DYSMD	YIYPNNGYTGYNQKFKSK	KLGRREAYFDV
51D1.1	DYYMA	NINYDGSSTYYLDSLKSR	TPTGYYAMDY

Clone	V_L		
	CDR1	CDR2	CDR3
12H5.1	RASKSVSTSSYSYMH	YASYLES	QHSREFPYT
36D1.1	KASENVVTYVS	GASNRYT	GQGYSYPYT
37C4.1	KSTQSLFNSRTRKNYLA	WASTRES	TQSYYLH
37C6.1	RASQDISNYLN	YTSRLHS	QQYSKLPYT
51D1.1	RASKSVDRYGNSFMH	RTSNLES	QQNNEDPWT

*Basic amino acid residues within CDR3 are shown in boldface.

Discussion

Since the discovery of spontaneous mouse models of SLE, it has long been debated if anti-DNA antibodies are mainly responsible for the development of fatal nephritis, or anti-gp70 antibodies reactive to the endogenous retroviral env gene product play more important roles in the formation of the glomerular injury. Pathogenic potentials of anti-DNA antibodies have been shown in various experimental settings, but recent genetic analyses have indicated a closer correlation between the production of gp70-anti-gp70 immune complexes with the development of glomerulonephritis than that between the anti-DNA antibody production and fatal nephritis. We have shown here that monoclonal anti-gp70 autoantibodies established from MRL/lpr lupus mice are directly pathogenic when transferred into non-autoimmune mice. The deposition of gp70 in affected glomeruli along with IgG and C3, and the development of severer lesions in NZW mice that express higher concentrations of serum gp70 than B6 mice that developed no significant pathology indicate that gp70-anti-gp70 immune complexes are involved in the development of the glomerular pathology.

The pathogenic anti-gp70 autoantibodies used the same VH gene family as known anti-DNA antibody clones, but possessed unique CDR3 sequences that are dissimilar to those of anti-DNA antibodies. Further studies are required to conclude if antigen-driven somatic mutations are accumulated in the VH and VL genes of the anti-gp70 clones as has been reported for anti-DNA antibodies [25,27].

Vascular lesions of necrotizing and/or granulomatous types are frequently observed in mice predisposed to develop systemic autoimmune diseases [6-8,14]. However, pathogenesis of spontaneous vascular injury has been largely unknown. In a previous study, we have shown a close correlation between the presence of anti-gp70 autoantibodies and the development of necrotizing arteritis in SL/Ni strain of mice [14]. Vascular lesions were also induced in mice transplanted with the pathogenic anti-gp70 antibody-producing hybridoma cells as we reported previously [28] and in the present study. The development of granulomatous arteritis by the injection of purified anti-gp70 autoantibody is a unique phenomenon, and this model promises to be useful for the analyses of pathogenetic mechanisms of and the development of therapeutic means for human inflammatory vascular diseases.

Our model of vascular injury induced by the injection of anti-retroviral autoantibodies also suggests that immune responses to human retroviruses might be involved in the development of human inflammatory vascular diseases. This is particularly of interest because infections with exogenous human retroviruses, HTLV-I and HIV-1, are known to be associated with vasculitis [8,9]. Although sceptical views are common among retrovirologists on the possible involvement of human endogenous retroviruses in the pathogenesis of autoimmune diseases [11,12], it might be worth recalling that most retrovirologists had been sceptical to the point of nearly denying the

possibility of retroviruses playing a role in human cancers until HTLV-I was finally discovered [1].

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Acknowledgements

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. We thank Mr. M. Patrick Gorman for critically reading and correcting the manuscript.

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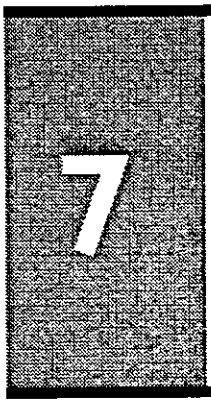
Recent Res. Devel. Virol., 6(2004): 105-118 ISBN: 81-7895-127-4



Host genes that influence immune and non-immune resistance mechanisms against retrovirus infections

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Abstract

Several host genes control retroviral replication and pathogenesis. These include host genes that directly affect the replication of retroviruses in target cells of infection and those affecting the host immune responses to the viral antigens. Host genetic factors that affect both retroviral replication and immune responses to the viral antigens have been best studied in mouse models of Friend leukemia virus (FV) infection. The presence or absence of an appropriate receptor restricts the attachment and entrance of viral particles into target cells, and the product of the Fv-4 gene blocks a receptor on mouse cells. APOBEC

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enzymes mutate reverse-transcribed viral DNA, and the Fv-1 gene product interferes with the proviral integration. Growth induction of infected erythroid progenitor cells is affected by the Fv-2 mutation of a hematopoietic cell growth factor receptor, STK. Several genes located within the major histocompatibility complex (MHC) and a separate gene not linked to MHC control the host immune responses. The latter, Rfv-3, controls the production of virus-neutralizing antibodies, and thus affects the duration of viremia. Both MHC class I-restricted $CD8^+$ and class II-restricted $CD4^+$ T cells are required for spontaneous and vaccine-induced immune resistance against Friend virus infection. Further, natural killer (NK) cells are activated soon after the viral infection, and are indispensable for vaccine-induced immune protection of susceptible mice. Single-epitope peptides that prime $CD4^+$ T cells are effective in inducing protective immunity against FV, and effector mechanisms including NK cells, $CD4^+$ and $CD8^+$ cytotoxic cells, as well as virus-neutralizing antibodies are involved in protection in the vaccinated animals. The above knowledge of the host genes that influence resistance against mouse retroviral infection may be useful when considering preventative and therapeutic means to human retroviral infections.

Introduction

Upon entrance into target cells retroviruses reverse-transcribe their RNA genome into double-stranded DNA, and the resultant viral DNA integrates into cellular chromosomes as proviruses. Integrated proviral DNA replicates through the process of normal cell division along with the cellular genome, and is transferred to daughter cells as a part of the cellular chromosomes. Since most retroviruses do not show cytopathic effects, productively infected cells continue to divide, supplying ever-increasing sources of infectious virus particles. Thus, for the elimination of retroviruses, the destruction of extracellular virions is insufficient. Rather, the eradication of the host cells that harbor integrated proviruses is required, making the control of retroviral infections more difficult than that of other viruses. In addition, the destruction of retrovirus-infected cells inevitably results in the loss of their functions, potentially causing harmful outcomes in the host body. Such harmful complications of the destruction of retrovirus-infected cells by the host's immune system are known as retrovirus-induced immunopathology, which is implicated in the development of acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus (HIV) infection, human T-cell leukemia virus type-I (HTLV-I)-associated myelopathy, a neurological complication of HTLV-I infection, and some autoimmune diseases associated with chronic retroviral infections [1-3]. In addition, if a retrovirus infects germ cells, the integrated provirus is transmitted through generations as a constituent of the host genome, resulting in the presence of viral genome in all the cells of each

progeny. Such germ line-transmitted proviruses are called endogenous retroviruses, and they are inherited in a Mendelian manner. More than 1% of the whole human genome is occupied by endogenous retroviruses or their remnants. There is no way for the host immune system to distinguish the products of endogenous retroviruses from those of other chromosomal genes, and thus the immune system has to ignore the endogenous retroviral proteins through the mechanism of self-tolerance.

Integration of a provirus into chromosomes can also cause a physical disruption of functional cellular genes, which may result in the loss of their functions. In addition, since viral long terminal repeats (LTR) are strong promoters of gene expression, proviral integration can cause the activation of adjacent cellular genes, which may lead to their overexpression and resultant gain of cellular functions. These mechanisms are known to be involved in retrovirus-induced tumorigenesis and to cause physically and/or functionally discernible mutations [4]. Thus, retroviruses are most threatening to the genetic integrity of all animal species. In this regard, it stands to reason that the existing mammals have evolved by acquiring a battery of mechanisms that restrict the infectious entry and replication of retroviruses. These include cellular mechanisms that interfere with the attachment and fusion of virions to target cells, including the presence or absence of cell-surface receptors, the mechanisms that affect the uncoating of viral nucleocapsid or reverse transcription, and those that interfere with the process of proviral integration. In addition, there are other mechanisms that work at the level of the whole body, particularly the immune responses directed toward the viral proteins and infected cells.

Friend murine leukemia virus and host genes that affect its replication and pathogenicity

Host mechanisms that affect the retroviral replication and pathogenicities have been most extensively studied in the mouse model of Friend retrovirus infection [5, 6]. Friend mouse retrovirus complex (FV) is composed of replication-competent Friend murine leukemia helper virus (F-MuLV) and defective spleen focus-forming virus (SFFV), the latter of which induces rapid growth and terminal differentiation of infected erythroid progenitor cells. FV is known to induce fatal erythroleukemia associated with severe immunosuppression when inoculated into immunocompetent adult mice of susceptible strains. One of the advantages of the FV system in analyzing host factors that affect retroviral replication and pathogenesis is that FV causes the rapid development of readily detectable splenomegaly associated with severe polycythemia, and ultimately causes the development of fatal leukemia within a few months after inoculation into adult mice. Most other retroviruses cause

neoplastic and/or neurological diseases only when they are inoculated into neonatal mice, and it usually takes more than 6 months for the virus-induced disease to develop. Inoculation of neonatal mice with an exogenous retrovirus causes immunological tolerance, and this induction of immune unresponsiveness and resultant persistence of viremia are crucial in the development of the above pathologies. On the contrary, FV can cause splenomegaly and fatal leukemia even when the virus is inoculated into immunocompetent adult mice, making the analyses of host immune responses and their modifications by preventative and therapeutic modalities possible. In addition, the presence of inbred strains has made the genetic analyses of host factors that affect FV infection much easier than retroviral infections of any other species. In fact, the FV system has led the genetic and molecular analyses of host factors in retroviral infections for decades, which have recently been applied and expanded to human retroviral infections. In the following sections, cellular factors that directly interfere with FV replication and FV-induced disease development are summarized, followed by a detailed description and discussion of the host genes that influence the immune responses against FV antigens. The possible implications of the knowledge acquired through the analyses of FV infection for the development of antiretroviral vaccines are also discussed.

Cellular genes that directly influence the replication of FV

Retroviral infection starts with the attachment of virions to the surface of target cells and the fusion of the viral envelope with the cellular membrane. These processes are mediated by the interaction between the viral envelope glycoproteins and cellular receptors. The presence or absence of appropriate receptors that can interact with the incoming viral envelope protein thus determines the infectivity of retroviruses to target cells. There are four known groups of mouse retroviruses that differ in their ranges of host specificities: ecotropic viruses infecting only mouse and rat cells, xenotropic infecting non-mouse cells, amphotropic infecting both mouse and non-mouse cells, and polytropic showing variable infectivities to mouse and non-mouse cells. Infectious polytropic viruses are recombinants between infectious ecotropic and endogenous polytropic viruses that carry varying segments of an endogenous polytropic viral *env* gene [7]. Viral envelope proteins of retroviruses that belong to the same host range group interfere with each other: thus, cells productively infected with an ecotropic virus cannot be superinfected by another ecotropic virus because of the receptor blockade. Xenotropic and polytropic viruses partly interfere with each other because they share the same polymorphic receptors. The cell surface receptor molecule for ecotropic viruses is mCAT1, a cationic amino acid transporter [8-10]. The receptor for

xenotropic and polytropic retroviruses is XPR1, and its polymorphism through different species affects the infectivities of recombinant polytropic viruses to host cells of different species [11-14]. The receptor for amphotropic viruses is separate from the above ecotrpoic and xenotropic viral receptors, and its molecular identity is that of an inorganic sodium phosphate transporter, Pit2 [15, 16]. A related transporter, Pit1, serves as the receptor for Gibbon ape retrovirus and feline leukemia viruses of cats [17]. One of the host genes that restrict the infection with FV, *Fv-4*, is an endogenous ecotropic viral *env* gene, the product of which directly interacts with mCAT-1 and blocks the attachment of incoming ecotropic virus [18, 19].

Once the virion attaches on an appropriate cellular receptor and the viral envelope fuses with the cell membrane, the process of reverse transcription starts immediately through the activation of the viral RNA-dependent DNA polymerase by the changes of pH and Mg^{++} or Mn^{++} concentrations and through the supply of nucleotide precursors. At this stage, cellular cytidine deaminases that are members of the apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like (APOBEC) family show broad antiretroviral activity. Thus, after the penetration of a retrovirus into target cells and the initiation of reverse transcription of the RNA genome into DNA, APOBECs can induce the conversion of cytosine to uracil in minus strand viral cDNA, leading to a failure of reverse transcriptase and to a very high number of G-to-A mutations in the integrated proviral genome that greatly reduces viral efficiency in its replication [20, 21]. Human APOBEC3G and APOBEC3F are shown to restrict the replication of HIV-1 as well as mouse retroviruses. HIV-1 Vif protein counteracts the activity of APOBEC3G by forming a complex with it in the cytoplasm and by impeding its packaging into virions, thus preventing editing mutations upon infection of the newly generated viral particles [20-22]. The interaction with Vif also stimulates APOBEC3G degradation by the ubiquitine-proteasome pathway, resulting in the increase in viral replication [23, 24].

The process of the integration of double-stranded viral DNA into cellular chromosomes is mediated by a complex of the double-stranded viral DNA and viral core proteins. The preintegration complex composed of the viral DNA, *gag*- and *pol*-encoded proteins, as well as some cellular proteins, is transported into the nucleus, and viral integrase mediates the nick formation in the chromosomal DNA and proviral integration. The product of host resistance gene *Fv-1* interferes with the process of proviral integration through the interaction with the preintegration complex [25]. *Fv-1* is the *gag* gene of a polymorphic endogenous retrovirus that belongs to a new member of mouse endogenous viruses (MuERV) similar to human endogenous retroviruses (HERV). Ecotropic mouse retroviruses are divided into two major groups depending on their infectivity to cells of two different strain groups: one

represented by cells derived from NIH Swiss mice (N-type), and the other represented by cells of BALB/c origin (B-type). N-tropic mouse retroviruses infect N-type cells more efficiently, while B-tropic viruses infect B-type cells more efficiently. The resistance is dominant, and thus (BALB/c × NIH Swiss)F_1 mice are resistant to both N-tropic and B-tropic viruses. Some tissue culture-adapted viruses can infect both N-type and B-type cells with comparable efficiencies, and are called NB-tropic. The exact molecular mechanisms of the action of *Fv-1* gene product are still unknown; interestingly, however, the same mechanism seems to work in human cells restricting the integration of mouse retroviruses [26].

Molecular mechanisms of Friend virus-induced disease development

Upon inoculation of FV complex, the virus replicates first in vascular endothelial cells, and then reaches hematopoietic cells of the bone marrow and spleen [27]. The product of the SFFV *env* gene, gp55, makes a complex with erythropoietin receptor (EpoR), and this interaction induces the growth and terminal differentiation of erythroid progenitor cells (Fig. 1). EpoR is expressed in erythroid cells at the stages later than burst-forming unit of erythroid (BFU-e) and is mainly effective in the growth regulation of the colony-forming unit of erythroid (CFU-e). The transduction of growth signals from the gp55-EpoR

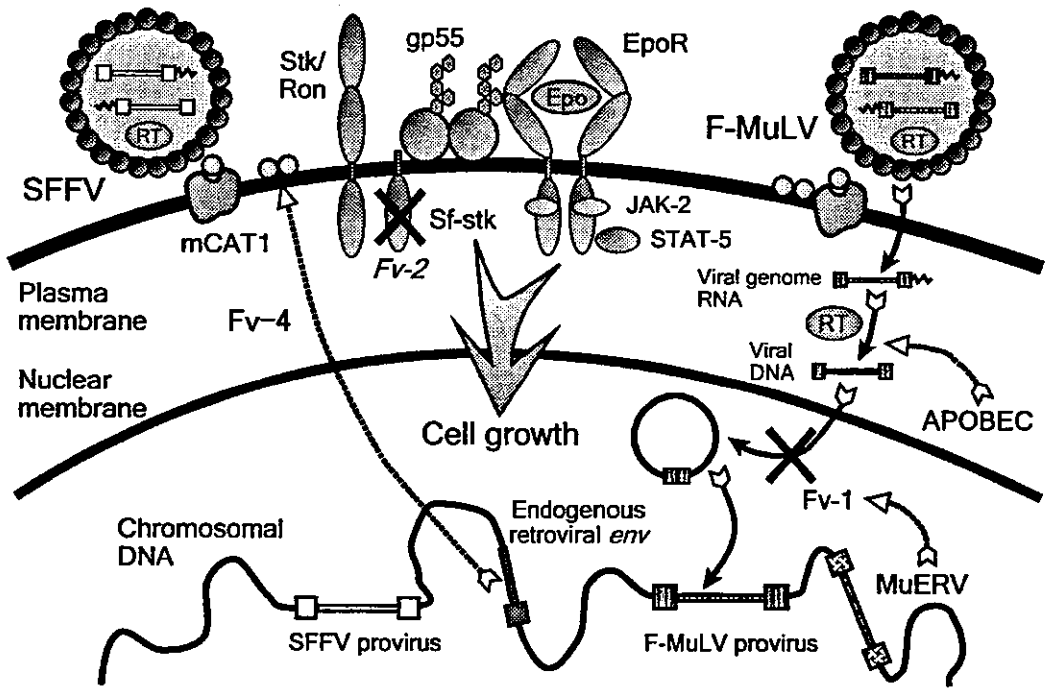

Figure 1. Schematic summary of the mechanisms of FV-induced erythroid cell proliferation and cellular factors that interfere with FV infection.

complex also requires the involvement of another molecule, sf-STK, which is a short form of a kinase-type hematopoietic growth factor receptor, STK [28, 29]. Due to mutations in the intron of the *STK* gene, mice of C57BL strains lack the expression of sf-STK, although they express the full-length STK. Due to this lack of sf-STK, C57BL/6 (B6) and C57BL/10 (B10) mice are resistant to FV-induced disease development. This genetic factor found in C57BL mice had been described as an FV-resistance gene, *Fv-2*. The recessive resistant allele $Fv\text{-}2^r$ is actually the above mutated *STK* that results in the lack of sf-STK.

It has been widely accepted that the SFFV-induced erythroid cell proliferation is crucial for the development of FV-induced erythroleukemia. That scheme of FV-induced leukemogenesis is as follows: upon infection of susceptible strains of mice with FV, SFFV gp55 induces rapid proliferation of erythroid progenitor cells. This leads to the development of splenomegaly and polycythemia readily detectable at as early as two weeks after FV inoculation. However, since the stimulation of erythroid progenitor cells through EpoR induces not only their growth but terminal differentiation, the process of erythroid cell expansion is self-limited, and progenies of SFFV-infected erythroid cells ultimately stop dividing and mature into red blood cells. Thus, even continuous infection of erythroid progenitor cells cannot result in the development of leukemia. For the development of malignant transformation, changes in the expression of host genes due to proviral integration into specific chromosomal locations are involved. Rapid expansion of erythroid progenitor cells is believed to provide target cells for repeated integration of SFFV and F-MuLV proviruses, and resultantly increases the chances for the proviruses to "hit" cellular genes by the promoter insertion adjacent to an oncogene or by disruption and silencing of a tumor suppressor gene [30, 31]. In fact, activation of several oncogenes and disruption of a few tumor suppressor genes have been documented in FV-induced leukemia cells. However, the proposed relationships between SFFV-induced early expansion of erythroid progenitor cells and malignant transformation due to proviral integration still remain circumstantial: for instance, it has not been proven if the SFFV-induced proliferation of erythroid cells is a prerequisite to allow repeated proviral integration resulting in a higher frequency of disease-inducing "hits." In this regard, it has been shown that even $Fv\text{-}2^{r/r}$ B6 mice can develop FV-induced leukemia when T lymphocytes are absent [32]. Further, our recent data suggest that SFFV-induced early splenomegaly is not a requisite for FV-induced leukemogenesis in a certain immunodeficient condition (Kawabata, H., *et al.*, manuscript in preparation). Thus, there are definitely interactions between host immune responses to FV-infected cells and the progression from the SFFV-induced erythroid cell proliferation to malignant transformation of virus-infected erythroid cells.

Host genes that control immune responses to FV infection

Even when mouse strains that share the same susceptible genotypes at the above host genes regulating FV replication and SFFV-induced cell growth are infected, the rate and tempo of the development of splenomegaly and leukemic death can change drastically depending on the host's genotypes at other genetic loci that regulate immune responses to FV. Fig. 2 shows examples of the effects of such immnunoregulatory genes. (B10 × A.BY)F_1 and (B10.D2 × A.BY)F_1 mice share the same (B10 × A)F_1 background, and thus share the *Fv-1*$^{b/b}$, *Fv-2*$^{r/s}$, and *Fv-4*$^{s/s}$ genotypes regarding FV infectivity. Nevertheless, (B10 × A.BY)F_1 mice are highly resistant to FV infection, and only <10% die within 100 days

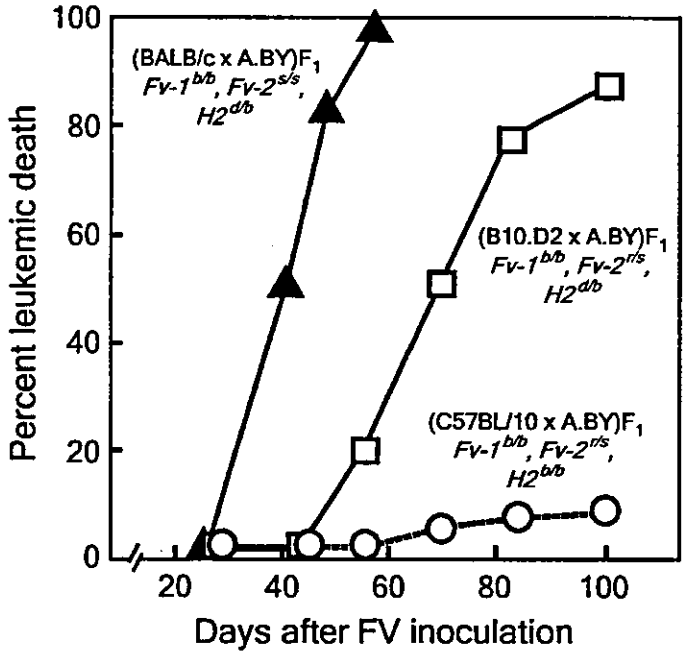

Figure 2. Comparison of tempos of leukemic death after FV infection in three different strains of mice.

after inoculation of a high dose of FV in the range of 1,500 to 30,000 spleen focus-forming units. These two strains differ only at the major histocompatibility complex (MHC) loci: (B10 × A.BY)F_1 possessing the homozygous *H2*$^{b/b}$ haplotype, and (B10.D2 × A.BY)F_1 being *H2*$^{d/b}$. Genes within mouse MHC that influence the incidence and tempo of the development of FV-induced splenomegaly and leukemic death have been mapped by utilizing H2 congenic and recombinant mouse strains [33-36], and are summarized in Table 1. Both class I-restricted $CD8^+$ and class II-restricted $CD4^+$ T cells are required for full resistance against FV-induced disease

Table 1. Host genes that influence immune response to FV infection.

Genes	Chromosomal location	Resistant allele	Susceptible allele	Phenotype influenced
Rfv-3	15	*Rfv-3*^{r} (C57BL)	*Rfv-3*^{s} (A/WySn)	Recovery from viremia, Kinetics of neutralizing Ab production
Rfv-1	17, *H2D*	D^b	D^d, D^k, D^q, D^{dm14}	Cytokine production from $CD8^+$ and $CD4^+$ T cells
Rfv-2	17, *Q/TL*	*Qa-1*^{a}	*Qa-1*^{b}	NK killing?
H2A	17	A^b	A^d, A^k, A^{bm12}	T helper cell responses to viral antigens
H2E	17	E^b (hybrid)	E^k, E^d	T helper cell responses to viral antigens

development. The requirement of both $CD4^+$ and $CD8^+$ T cell subsets for the spontaneous immune resistance of $H2^{b/b}$ mice was further substantiated by the results of antibody (Ab)-mediated T cell depletions [37]: the otherwise resistant mice depleted of $CD8^+$ T cells developed rapid splenomegaly, while the same strain of mice depleted of $CD4^+$ T cells showed initial recovery from splenomegaly, but ultimately developed leukemia and died at a high frequency. Further, administration of Ab reactive to the class II E molecule, but not those reactive to the A molecule, abrogated the spontaneous resistance against FV infection [38].

Interestingly, mice lacking the resistance-associated *b* allele at the class II loci as well as those depleted of $CD4^+$ T cells nevertheless showed spontaneous recovery from the initial development of splenomegaly. These results suggest that functions of $CD8^+$ T cells are required in the earlier, and those associated with $CD4^+$ T cells in the later stages of the infection, together exerting immune resistance against FV infection.

Epitopes recognized by $CD4^+$ and $CD8^+$ T cells have been identified in the *env* and *gag* gene products of F-MuLV [39-46], and are summarized in Fig. 3. Among these, an *env*-encoded, class II $E^{b/d}$-restricted peptide VYSQFEKSYRHKR and a *gag*-encoded class II-restricted peptide IVTWEAIAVDPPP identified within the matrix protein (MA) were effective in inducing protective immunity against FV challenge when given as a vaccine to $H2^{a/b}$ or $H2^{d/b}$ strains of susceptible mice [44, 47, 48]. In cases of both these peptides, virus-neutralizing Ab were not detectable before FV challenge, although non-neutralizing anti-MA Ab were detectable in mice immunized with the gag peptide. Rapid production and IgM-to-IgG class-switching of virus-neutralizing Ab were observed in peptide-immunized mice after FV challenge, indicating an advantage of $CD4^+$ T cell-priming vaccines in facilitating virus-reactive Ab production. Effector cells including $CD8^+$ and $CD4^+$ cytotoxic T lymphocytes (CTL) and natural

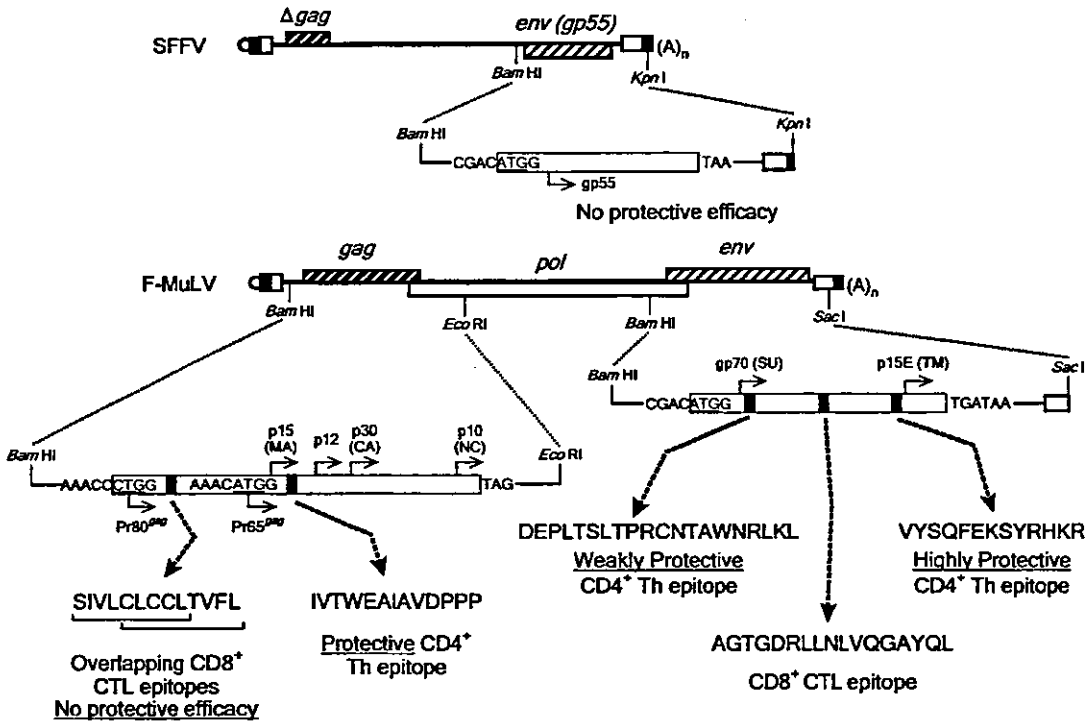

Figure 3. Amino acid sequence and location of the epitopes recognized by T cells in FV gene products.

killer (NK) cells are activated in mice immunized with the *env*-encode peptide within a few days after FV inoculation [48]. The $CD4^+$ CTL was only detectable in the peptide-immunized mice, but $CD8^+$ CTL and NK cells are activated both in immunized and unimmunized animals after FV infection. Further, NK cells were most efficient in killing FV-infected target cells, and Ab-mediated depletion of NK cells totally abrogated the protective efficacy of the *env*-encoded peptide vaccine [48]. These results indicate that peptide-induced priming of $CD4^+$ T cells augments the activation of multiple effector mechanisms upon FV infection. There has so far been no evidence showing that immunization with a single $CD8^+$ T cell epitope induces protection against FV infection. Further, immunization of mice with a recombinant vaccinia virus that expresses the SFFV *env* gene did not induce protective immunity [49], suggesting the lack of immunoprotective epitopes within the SFFV *env* gene products.

Another MHC-associated host gene that influences the development of FV-induced splenomegaly has been mapped in the class Ib Q/TL loci [35]. The exact molecular nature of this resistance gene, *Rfv-2*, has not been identified, but given the involvement of a Q-locus gene product, $Qa\text{-}1^b$, in the negative regulation of the target cell killing by NK cells [50], and the observed importance of NK cells in protective immunity against FV, it is possible that *Rfv-2* is actually *Qa-1*, and is regulating the susceptibility of FV-infected cells to NK killing.

Rfv-3 gene that controls the production of virus-neutralizing antibodies

The results shown in Fig. 2 also indicate that, although (B10.D2 × A.BY)F_1 and (BALB/c × A.BY)F_1 strains of mice share the same $H2^{d/b}$ haplotype, they differ completely in their tempo of leukemia development after FV infection. In fact, (BALB/c × A.BY)F_1 mice die within 2 months after FV infection, while nearly half of (B10.D2 × A.BY)F_1 mice survive through post-infection day (PID) 70. The association between genotypes at a non-MHC host gene and the development of FV-induced disease was first described for the persistence of viremia after FV infection [51]: A/WySn mice persisted to be viremic at > 30 days after FV infection, while B10.A mice had cleared viremia by PID 30. Since F_1 crosses between these two strains were not viremic at PID 30, and about half of the (B10.A × A/WySn) × A/WySn backcross mice showed viremia at the same time-point, the presence of a recessive host gene was postulated in association with the persistence of viremia and was designated as *Rfv-3*^{s}. Thus, B10 mice possess a dominant allele, *Rfv-3*^{r}, conferring the early clearance of viremia. The *Rfv-3* locus was later mapped within chromosome 15 [52, 53]. To clarify the relationships between the *Rfv-3* genotypes and the production of virus-neutralizing Ab, we performed genetic mapping experiments by using > 200 (B10.A × A/WySn) × A/WySn backcross mice (Kanari, Y., et al., submitted for publication). A single gene determining the production of F-MuLV-neutralizing serum Ab at PID 15 was mapped in chromosome 15, and the strongest association was observed between the neutralizing titers at PID 15 and genotypes at the D15Mit71 locus, colocalizing with the previously mapped *Rfv-3* locus (Fig. 4). There are a few candidate genes that might be responsible for the observed regulation of neutralizing Ab production. Of note, A/WySn, but not A/J, mice are known to possess a mutation in the B cell-activating factor belonging to the TNF family receptor (BAFF-R) gene that results in the attenuation of germinal center responses of antigen-simulated B lymphocytes [54]. The structural gene for the BAFF-R is located in chromosome 15, a few megabase pairs (Mbp) telomeric to the D15Mit71 marker. In addition, the structural gene for mouse APOBEC3, the anti-retroviral enzyme, is also mapped in the same chromosome, located a few Mbp centromeric to the same D15Mit71 locus. Although there is no documented direct influence of APOBEC molecules on the functions of B lymphocytes, a presumably reduced replication of FV in the early stages of infection in the presence of a resistant APOBEC genotype, if any, might allow the host immune system to mount stronger Ab responses while infectious virions are still small in number. The possible presence of polymorphisms in the APOBEC3 locus between the resistant and susceptible strains must be demonstrated before making any further assumptions on the

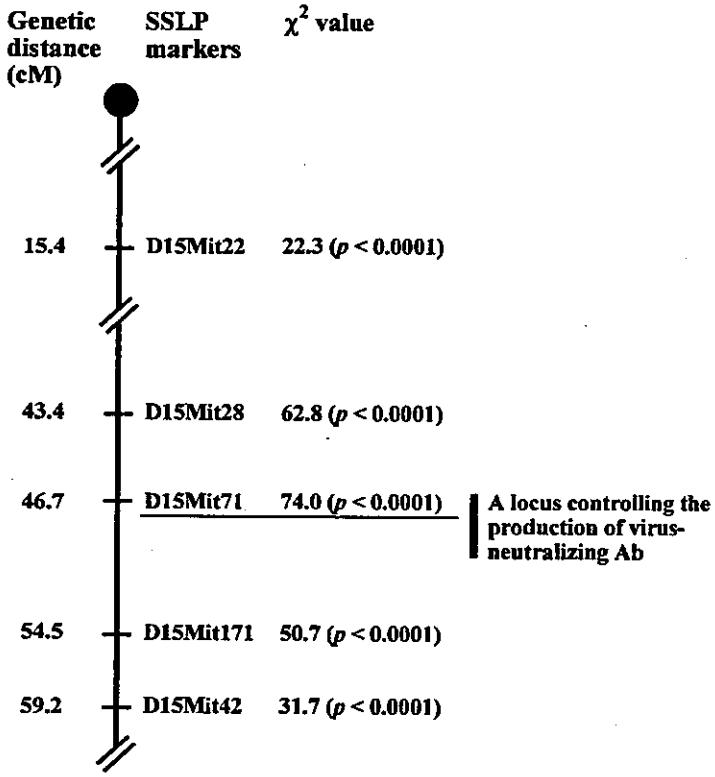

Figure 4. Genetic mapping of the host gene that controls serum titers of virus-neutralizing Ab in FV-infected mice.

role of this gene in the regulation of neutralizing Ab production. Additionally, the possible direct effect of the known polymorphism of the *BAFF-R* gene on the production of FV-neutralizing Ab can be assessed in the near future by establishing transgenic mice that express the B10.A-derived BAFF-R gene on the background of A/WySn strain.

Acknowledgements

The author's work documented in this review has been partly supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology, and the Ministry of Health, Labor, and Welfare of Japan, and those from the Japan Health Science Foundation. The author thanks Mr. M. Patrick Gorman for his critical review and correction of the manuscript.

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