Evidence Supporting a Physiological Role for Endogenous C-Type Virus in the Immune System

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The discovery that sequences coding for C-type viruses were endogenous to the genome of a variety of vertebrates raised several important questions. Principal among these were the relationship of endogenous viruses to oncogenic C-type viruses and their putative function during normal differentiation and development of their host cells. Recently a possible link between endogenous virus expression and the development of spontaneous leukemia has been proposed by the work of Hartley and Rowe with AKR mice [1]. Reasons for the retention and expression of these viruses in normal cells, however, have been lacking.

A few years ago we reported that several B-cell mitogens induce the expression of endogenous virus in murine spleen cell cultures [2,3]. These mitogens (lipopolysaccharide *E. coli*, tuberculin, lipoprotein *E. coli*) also trigger DNA synthesis and the differentiation of precursor B-cells into immunoglobulin-secreting end cells [4]. Recently we have shown that with hot thymidine suicide and inhibitor experiments, the stimulation of DNA synthesis is a prerequisite for virus production [5]. With these facts in mind, we began an examination of the molecular mechanism of virus induction as well as the relationship between virus expression and the immune response. Here we discuss these data in light of our hypothesis that the expression of endogenous viral antigens plays a physiological role in the humoral immune response of mice.

The molecular mechanism by which mitogens induced virus was investigated by assaying for viral transcripts in lymphocytes. DNA complementary to viral RNA was synthesized by the viral endogenous reverse transcriptase and used as a virus-specific probe. Hybridizations between control lymphocyte RNA and cDNA showed a low level of viral transcripts, homologous to approximately 25% of the probe (Table 1). This expression of viral sequences in unstimulated control lymphocytes was found both with BALB/c cells, which release virus following mitogen stimulation, as well as with cells from 129 mice, which do not release virus. BALB/c lymphocytes treated with mitogens increased their expression of virus sequences significantly (Table 1). We conclude from these results that expression of viral sequences and, presumably, viral protein(s) is a trait common to murine lymphocytes and that additional sequences are synthesized in response to mitogen induction.

The fact that mitogens mimick antigenic stimulation led us to hypothesize that virus expression may reflect a physiological process necessary in the gen-

Table 1. Endogenous viral transcripts in murine lymphocytes [9]

Source of RNA	Treatment	Saturation hybridization values[a]
Indicator fibroblast line	control	8%
	virus producing	70%
BALB/c spleen cells	control	23%
	+ LPS/BU[b]	55%
129/J spleen cells (uninducible strain)	± LPS/BU[b]	25%

[a] DNA complementary to induced virus was hybridized to saturation with the indicated RNAs in excess RNA reactions.
[b] Lipopolysaccharide E. coli in combination with bromodeoxyuridine

eration of immunoglobulin-secreting cells. We tested this hypothesis by injecting mice with a rabbit antibody directed against endogenous virus. Such animals showed reduced numbers of immunoglobulin-secreting cells following antigenic stimulation [6,7]. Experiments with this immunosuppressive serum are summarized in Table 2. Immunosuppression was observed with different antigens, in all mouse strains tested, and was also observed in in vitro systems. Absorption experiments confirmed the viral specificity of the immunosuppressive component. Since $F(ab')_2$ fragments were equally effective in immunosuppression, cytotoxicity can be ruled out as the mechanism of immunosuppression. Systems measuring cellular immune responses were, however, not affected. Surprisingly, the antibody was only immunosuppressive when administered early during the 4–5 day immune response against sheep red blood cells. This suggested that it interfered with the events which lead to triggering the B-cells to divide, possibly in T–B-cell cooperation. This conclusion was supported by recent experiments using the KLH-DNP carrier-helper system, performed in collaboration with Dr. Peter Erb, which revealed that activated T-helper cells were one target of the serum. A second target was the B-cell, since the serum also suppressed the T-independent anti-

Table 2. Immunosuppression by rabbit antiserum directed against BALB/c endogenous xenotropic virus

		References
Response suppressed:	sheep red blood cells, horse red blood cells, KLH-DNP, non-soecific polyclonal anti-DNP	6, 7 and unpublished results with Dr. P. Erb
Strains suppressed:	BALB/c, C57BL, AKR, DBA, 129	7
Specificity:	effect absorbed by purified virus, no effect on cellular immunity	6, 7 and unpublished results with A. Brownbill
Mechanism:	independent of complement, acts early during the immune response, possible blocking of T–B-interaction	7 and unpublished results

DNP response against bead-coupled DNP (unpublished results). These results are consistent with data from Wecker and coworkers who demonstrated the presence of viral glycoprotein gp70 on T- and B-cells participating in an anti-KLH-DNP response [8].

It was concluded that blocking of viral structures present on T-helper cells and antigen reactive B-cells suppresses the immune response. Two alternative mechanisms are consistent with our data. Blocking of viral antigens could lead to steric hindrance of a functional structure. Alternatively, viral antigen itself may represent a structure necessary for the generation of a humoral immune response. The present data do not allow us to distinguish between these explanations. However, the hypothesis that endogenous virus plays a physiological role remains a plausible interpretation.

Our studies on the induction of endogenous virus in mouse lymphocytes have established several features of this phenomenon. When mitogens stimulate B-cells of inducible mouse strains to synthesize DNA and differentiate, endogenous viruses are activated. Virus induction requires cellular DNA synthesis in the stimulated cells. These lymphocytes then transcribe new viral sequences not previously found in the population. However, unstimulated cells contain viral sequences that may represent memory cells as yet unactivated.

The expression of endogenous viral antigens on lymphocytes does not appear to be fortuitous since antisera against these viruses block humoral immune responses. These data are consistent with viral antigens mediating B-cell activation either through a T-cell interaction or directly. On T- and B-cells for example, viral antigens could be involved in cell-cell recognition. Alternatively, they could be secreted from the T-helper cell and deliver a biochemical signal necessary for B-cell activation. Should this hypothesis prove correct, it is conceivable that understanding how viral genes function in lymphocyte activation will also shed light on how leukemia viruses are involved in cell transformation.

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