enzyme. After closure, the low molecular weight products that are generated increase to a concentration that favors movement across the membrane by a carrier. Thus, caveolae could act transiently to form vesicular compartments that protect either the substrate or the product and thereby control the direction of molecular movement to benefit the cell.

Caveolae may also control the transport of small molecules into cells by housing a receptor that could bind an extracellular carrier protein bearing a low molecular weight ligand. The caveola would close, the pH or some other parameter would change, and the ligand would be released. Transport across the membrane into the cytoplasm would occur as the concentration increased. Potocytosis offers the cell a different kind of control over the spatial and temporal uptake of the ligand than does receptor-mediated endocytosis, which involves extensive membrane traffic through the cellular endomembrane system.

Another potential function for potocytosis is to receive or to transmit various kinds of cellular signals, such as signaling molecules derived from GPI-anchored membrane molecules. It has been proposed that insulin (*17*), nerve growth factor (*18*), and interleukin-2 (*19*) transmit messages to cells in part through inositol phosphoglycan (IPG) intermediates that are generated from GPI-anchored membrane proteins or lipids. All GPI-anchored molecules appear to be located on the external surface of the plasma membrane (*20*) so that any released IPG would rapidly diffuse away from the cell. Caveolae could solve this problem by controlling the transmembrane movement of either IPG or its metabolite if both the GPI-anchored molecule and an appropriate phospholipase were housed in this compartment. Insulin receptors have been found to be located near adipocyte caveolae (*21*), which place them in an optimal position for stimulating a reaction cascade that leads to the import of IPG intermediates.

As a result of studies of folate internalization and bulk-phase sequestration by caveolae (*16*), certain characteristics can be predicted of a membrane-bound molecule suspected of being involved in potocytosis. (i) The molecule should be associated with caveolae. (ii) Internalization and recycling of this molecule should occur with a cycle time of about 1 hour. (iii) Internalization ought to be relatively temperature-insensitive, as compared to internalization by clathrin-coated pits. (iv) The molecule should not be degraded after internalization. The folate receptor (*3–9*), 5′-nucleotidase (*22*), alkaline phosphatase (*23*), cholera toxin (*24*), and lipoprotein lipase (*25*) are examples of membrane-associated molecules that meet one or more of these criteria. Further work is required to determine if these molecules are involved in potocytosis.

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Superantigens and Endogenous Retroviruses: A Confluence of Puzzles

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A SPECIAL PLEASURE OF DOING SCIENCE COMES WHEN long-standing problems in seemingly unrelated areas flow together into a single problem. The association between certain retroviruses—the mammary tumor viruses—and superantigens is one such event that not only provides a solution to an old immunological puzzle and partially solves a virological one, but promises to provide new insights into the interaction of viruses with the immune system.

T cells can be activated or depleted independently of conventional antigens (*1–3*). Bacterial superantigens comprise a set of bacterially produced protein toxins that, when complexed with major histocompatibility complex (MHC) class II molecules, specifically bind to certain subsets of T cell receptor (TCR) β chains (*4*). This binding causes stimulation of T helper cell function as though the cell had been exposed to antigen in the context of a presenting cell.

Endogenous superantigens were first identified as genetic elements that could stimulate T cell proliferation in mixed cultures of lymphocytes from mice that were matched at the MHC. The genes responsible for this effect were named *Mls* (for minor lymphocyte stimulating), and several distinct loci have been identified. Like bacterial superantigens, Mls stimulates only T cells that express a particular one (or a subset) of the 20 or so genes encoding alternative β chains of the TCR (Table 1). But like normal antigens, stimulation requires presentation of the *Mls* product together with MHC class II proteins on the surface of antigen-presenting cells. During development, mice that have *Mls* actively delete those T cells that express the V_β subset or subsets that recognize the specific *Mls* product. The genetics of *Mls* were initially confusing, but eventually it was recognized that there are several distinct *Mls* loci, each of which has two alleles: a positive allele that confers a specific pattern of V_β reactivity and a negative or null allele that confers no reactivity. Although the properties of Mls proteins had been inferred

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from indirect experimentation, their biochemical characterization had not yet been accomplished.

The known exogenous mouse mammary tumor viruses (MMTVs) are all vertically transmitted agents passed from mother to offspring in milk. As a side effect, the virus is capable of inducing mammary carcinomas and has provided a useful source of new oncogenes (*5, 6*).

The proviral DNA of MMTV (*7, 8*) has a long terminal repeat (LTR) at either end of the DNA that contains an open reading frame well conserved among strains. It is capable of encoding a protein of about 320 amino acids (Fig. 1), referred to as the orf (open reading frame) protein and has eluded attempts to identify its function. Although an appropriately spliced mRNA can be found, its product has been sighted only twice (*9*): in a partially deleted form in a T cell tumor and expressed from a baculovirus vector. The orf protein appears to be unnecessary for viral replication. The obvious idea that it might be involved in activation or regulation of MMTV expression has not panned out although some recent work ascribes a slightly suppressive effect on expression (*10*).

As is true for several retroviruses, MMTV can also be found as endogenous proviruses in the germline of inbred and wild mice (*11*). About 30 such proviruses—some complete, some defective—have been identified and mapped to specific chromosomal locations (*12*). At least two of the proviruses (*Mtv*-1 and *Mtv*-2) can yield infectious virus. Different strains of mice have Mtv proviruses inserted at different genomic locations, but the Mtv sequences from one strain to another are closely related, with little polymorphism among proviruses at the same locus. Thus individual proviruses have been recently inserted by infection of germline cells.

One final aspect of MMTV biology is the induction of T cell lymphoma, an unusual sequela of milk-borne infection with exogenous (*13*) or endogenous *Mtv* (*14*). In all cases the virus causing the T lymphoma has suffered a characteristic deletion encompassing a significant portion of the LTR (*15*) (Fig. 1) that includes most of the glucocorticoid responsive elements, a negative regulator of expression in T cells (*16*), and a substantial fraction of the COOH-terminus of the orf protein. Although all the mechanisms involved in lymphoma induction have not been fully worked out, the result demonstrates the interaction between MMTV and T cells.

The first glimmer of an association between the virological and immunological phenomena was the observation of genetic linkage of "*Mls*-like" activity to an endogenous *Mtv* provirus (*17*) (Table 1). Indeed, all described *Mls* loci are now known to be genetically inseparable from endogenous *Mtv* loci (*18–20*). Also, a new *Mtv* provirus, introduced as a transgene, confers an *Mls* phenotype (*21*).

Table 1. MMTVs and superantigens. The first two viruses listed are the milk-borne viruses passed from mother to offspring in the indicated mouse strains; the remainder are endogenous proviruses, each inherited by some but not all strains of inbred mice.

MMTV	V_β specificity	Reference
	Exogenous	
C3H	14, 15	(*22, 24*)
GR	14	(*21*)
	Endogenous	
Mtv-7 (*Mls*-1)	6, 8.1, 9	(*19*)
Mtv-"MA"	6, 8.1, 9	(*19*)
Mtv-1	3	(*19*)
Mtv-6 (*Mls*-3)	3	(*19*)
Mtv-13 (*Mls*-2)	3	(*19*)
Mtv-9	11	(*17, 18, 20*)
Mtv-8	5.1, 5.2, 11	(*20*)
Mtv-11	11	(*20*)

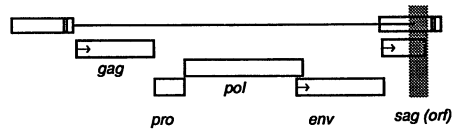

Fig. 1. The genetic organization of MMTV. The boxes in the proviral DNA (top line) indicate the LTRs; coding regions are indicated below. The shaded region approximates the deletion found in viruses that induce T cell tumors.

In addition, mice that carry maternally transmitted or deliberately introduced MMTV also display an *Mls*-like deletion of the $V_\beta 14$ subset (*22*).

Further proof of this association has been provided by direct tests in vitro. Introduction of the 3′ half of the cloned *Mtv*-7 provirus into an *Mls*-1–negative B cell line induces *Mls*-1–like activity, as measured by stimulation of interleukin-2 production after mixing with an appropriate T cell line (*23*). Similarly, cloned exogenous MMTV DNA stimulates $V_\beta 14$ T cells in vitro and causes their deletion in vivo (*24*). In both cases, the *orf* region alone in an appropriate expression vector is sufficient to confer activity. Although there may be complications related to control of expression of the gene, *Mls* activity is clearly due to the expression of its gene product, which most likely interacts directly with the appropriate V_β chain of the TCR.

The *orf* regions from different endogenous and exogenous MMTV proviruses are closely related to one another, with the greatest divergence at the COOH-terminus (*24, 25*). The COOH-terminal sequence covaries with V_β specificity, but the implication that this end of the molecule contains the V_β-interacting region remains to be tested directly.

Because of the association of the *orf* region with superantigen activity and the evidence of functional significance (below), I propose that *orf* be renamed "*sag*." This is similar to the suggestion of Choi and co-workers (*24*), but in agreement with conventions for naming retroviral genes (*26*).

It has been postulated that superantigens may be beneficial to the organism by depleting subsets of T cells whose presence might otherwise put the animal at risk for toxic effects of bacteria producing exogenous superantigens (*1, 24*). However, the present observations make it very difficult to sustain this or most other hypotheses based on a generalized beneficial effect of early removal of T cell subsets. To date, the endogenous superantigens are associated only with endogenous *Mtv* proviruses. Since these are found only in mice (and only in a few of the many species and subspecies at that), this can hardly be a phenomenon of general importance among mammals. Indeed, careful examination of the V_β chain usage in the T cell repertoire of other mammals fails to provide consistent evidence for distortion in subset usage (*27*), but the issue is still rather controversial. Most probably, the functional explanation for this curious effect lies with its value to the virus, not the host.

At present we can only speculate on what value superantigen activity might have for the virus. That the phenomenon is not due to chance interaction is implied by the conservation of the function even in the face of diversity in V_β specificity. The gene that encodes the superantigen is unique to MMTV; no such gene is present in any other known retrovirus, including the closely related D-type virus genus (*28*).

The replication of many (perhaps all) retroviruses seems to require that the infected cell be actively dividing at the time of infection. Because transfer of virus from the site of primary infection (the gut) to the site of transmission (the mammary gland) requires infection of T cells (*29*), stimulation of these cells into cycle coincident with the entry of the virus might be expected to increase the efficiency of infection. This effect would be simplest to visualize if the sag protein were on the surface of virions and active as a

superantigen in this context, but transfer of virus from one infected cell to another could also be promoted. [$CD8^+$ T cells can also present *Mls* in vivo (*30*).] Although many DNA viruses can stimulate division of infected cells, only some retroviruses are known to do so (*31*). Indeed, the role of the presenting cell remains to be clarified.

Another possibility is that the sag protein serves to prevent T cell lymphoma induction. The variant LTR in the T cell tumor-inducing viruses invariably lacks the region encoding the COOH-terminus of the sag protein (Fig. 1); thus, the presence of a complete gene might be inimical to tumor formation—perhaps due to elimination of Mls-expressing cells. Alternatively, the deleted form of the protein might retain some sort of unregulated stimulatory activity that could contribute directly to the oncogenic process.

One of the most striking aspects of the *Mls* phenomenon is variation in V_β recognition-specificity that is induced by closely related viruses, reminiscent of the variability in receptor usage exhibited by the envelope proteins of some groups of otherwise closely related retroviruses (*32*). We do not know the selective basis of this variability. Given their short time of residence in the germline and very low mutation frequency, it is improbable that selection acting on the endogenous proviruses could be important for the generation of diversity of V_β recognition. However, if exogenously transmitted MMTV becomes established as a germline provirus, it becomes an *Mls* gene with the same reactivity as the parental virus. The resultant removal of cells expressing that V_β subset would leave the T cells of the mouse less likely to be infected by the exogenous virus early in life. Thus, the endogenous provirus might confer a selective advantage on the mouse that carries it by inducing resistance to the pathogenic effects of exogenous MMTV and also put a strong pressure on an infecting exogenous virus of the same reactivity. This could lead to rapid selection of variants of exogenous virus specific for other V_β genes.

Although there is as yet no evidence that any of the numerous endogenous murine leukemia viruses (MLVs) encode superantigen-like activity, a variant exogenous MLV, known as LP-BM5 (or MAIDS, murine acquired immunodeficiency syndrome, virus), induces an immunodeficiency disease involving loss of T cells due to expression of a variant virion (gag) protein on infected B cells (*33*). B cell tumors infected with this virus also express a superantigen-like activity, which may be encoded by the variant protein (*34*). Thus the realm of virus-cell interaction may include other examples of stimulation of cells of the immune system to the advantage of the infecting virus. For example, it is possible that the pathogenesis of AIDS involves a superantigen effect of an HIV (human immunodeficiency virus) gene product (*2, 35*). Also, the sequences of the *Mtv* sag protein and a protein encoded by Herpesvirus saimiri, a virus that causes T lymphoma in monkeys, are strikingly similar (*36*). Without doubt, other interesting ways in which viruses mimic immunological signals for their own advantage remain to be discovered (*3, 37*).

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