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Sleeping with the Enemy—Endogenous Superantigens in Humans

Minireview

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We usually think of superantigens (SAg) as dangerous toxins that may cause toxic shock syndrome and death (Marrack and Kappler, 1990). Now, based on two papers in this issue of *Immunity*, it seems that we all have SAg genes within us, lying dormant and waiting to be activated under special circumstances (Sutkowski et al., 2001; Stauffer et al., 2001).

SAg are a group of microbial proteins that target the immune system, causing massive polyclonal T cell activation and cytokine release, often followed by T cell apoptosis or anergy. SAg differ from conventional peptide antigens. They are not processed into small peptides by antigen-presenting cells, and they bind to MHC class II outside of the peptide groove. They interact with the TCR in an unusual way, via amino acid residues encoded by the Vβ genes of the TCR rather than with the highly variable, non-germline-encoded residues of the TCR β and α chain CDR3 regions. Thus, large percentages of T cells expressing the same Vβ genes are stimulated.

Mouse Mammary Tumor Virus Superantigens

Endogenous *sag* were first discovered by Festenstein in 1973 and referred to as "minor lymphocyte-stimulating" (Mls) antigens. Mls antigens expressed on B lymphocytes were able to induce massive T cell proliferation in mixed lymphocyte reactions between MHC-identical strains of mice. Much later, it was shown that Mls genes were encoded by genomic copies of endogenous *sag* genes that had been pirated from the exogenous genomes of mouse mammary tumor viruses (MMTV) (reviewed in Acha-Orbea and MacDonald, 1995). The *sag* gene, it turned out, partly overlapped with the 3′LTR of MMTV in a location similar to the *nef* gene of HIV. Each MMTV-encoded SAg varies in the C-terminal region, conferring the TCR-Vβ specificity of this type II protein.

The biological function of *sag* genes was revealed in an elegant experiment (Golovkina et al., 1992). C3H mice transmit an exogenous MMTV vertically from mothers to pups. The *sag* gene from this virus was used to create transgenic mice that overexpressed the SAg during thymic maturation and, as a result, deleted the targeted Vβ14-bearing T cells. These mice were resistant to infection with exogenous MMTV. These studies were extended (Held et al., 1993) with the discovery that infection by exogenous MMTV from maternal milk was critically dependent on T cell help provided by the SAg-targeted Vβ subset. Due to the presence of SAg, initial amplification of MMTV RNA was on the order of 10^5–10^6. This amplification was in part due to more efficient viral production and in part due to the proliferation of infected B cells (Held et al., 1994). There was no evidence for a SAg-independent pathway of MMTV transmission and only MMTV with functional *sag* genes could be transmitted through milk (Golovkina et al., 1995). Thus, the life cycle of exogenous MMTV was dependent on SAg expression. In general, optimal replication of retroviruses requires actively dividing host cells. MMTV-encoded SAg appeared to stimulate the immune system (T and B cells) to provide such conditions.

On the other hand, it was suggested that *sag* encoded by an endogenous provirus conferred a selective advantage on the mouse that carried it. The responding Vβ subsets were deleted or became tolerant to the self-(super)antigen. As a result, these mice could avoid infection with exogenous MMTV that encode *sag* with the same Vβ specificity. If this was a successful evolutionary strategy to prevent infection with exogenous retroviruses in mice, one might expect that other species would have integrated similar endogenous *sag* genes. Until now, there was little evidence for this.

Endogenous Superantigens in Humans

A provocative paper (Conrad et al., 1997) provided the first evidence of possible endogenous human SAg. Conrad and colleagues were studying type I diabetes and found high percentages of Vβ7 T cells infiltrating the pancreatic islets in several patients. This was attributed to endogenous SAg encoded by the *env* gene of a human endogenous retrovirus (HERV), initially named IDDMK$_{1,2}$22 and later found to be identical to HERV-K18. This SAg was specific for Vβ7 T cells. In addition, viral RNA was found in plasma samples from newly diagnosed IDDM patients. However, other groups were unable to reproduce these findings (Murphy et al., 1998; Lower et al., 1998; Lan et al., 1998; Lapatschek et al., 2001).

An important lead was provided when HERV-K18 was found to be located within the first intron of CD48 on chromosome 1 (Hasuike et al., 1999). The group of Huber had been studying a SAg-like activity associated with EBV infection (Sutkowski et al., 1996), and CD48 expression was known to be induced in EBV-infected B cells, with an identified upstream EBV-inducible enhancer.

In this issue of *Immunity*, this group shows that the previously described EBV-related SAg activity is in fact encoded by alleles of the HERV-K18 *env* gene (Sutkowski et al., 2001). The more common K18.1 and K18.2 alleles were both found to encode *sag* genes specific for TCR Vβ13 and Vβ9. EBV infection led to transcriptional activation of HERV-K18 *env* and the EBV-associated SAg activity was blocked with an antiserum to HERV-K18 *env*. In the other paper from Conrad's group (Stauffer et al., 2001 [this issue]), three alleles of HERV-K18 *env* were identified and were discriminated from other KERV-K provirus genes based on their insertion site within the CD48 intron, an achievement that was essential for this work. All three had SAg activity and stimu-

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lated primarily Vβ7 (and possibly Vβ13.1) T cells. K18.1 was identical to IDDMK$_{1,2}$22 and contained a stop codon at residue 153, while K18.2 and K18.3 encoded the full-length protein. Thus, the SAg activity seems to be contained within the N-terminal ≈150 amino acids. Expression of the HERV-K18 alleles was strongly induced by αIFN treatment of PBL. Vβ7-specific SAg activity was inhibited with specific antisera to *env* peptides. Induction of HERV-K18 RNA by αIFN occurred in $CD2^-$ cells (which include B cells) but not in $CD2^+$ cells.

The two papers together suggest that EBV, and perhaps other viruses, can induce endogenous *sag* genes encoded by HERV and that type I interferons may be partly responsible for *sag* gene activation. However, there are still a number of loose ends. Differences concerning the Vβ specificity observed in the two papers need to be resolved. Even though both groups used the same A20 transfectants, the SAg was specific for Vβ13 and Vβ9 in one study (Sutkowski et al., 2001) and Vβ7 (and perhaps Vβ13) in the other study (Stauffer et al., 2001). Differences in the methods used might be relevant. For example, Sutkowski et al. treated their EBV lymphoblastoid cell lines and their transfected cell lines with PMA. Stauffer et al. used αIFN to induce PBL-derived APCs, and their A20 transfectants did not require pretreatment. There are seven *BV13*, three *BV7*, and one functional *BV9* gene. Some are known to have allelic variants. These various Vβ family members and even individual TCR Vβ alleles, which differ at crucial amino acid residues (Liao et al., 1996), may interact differently with SAg. Which of these gene products react with the HERV-K18 SAg has not yet been worked out. Finally, the polyclonal background T cell activation observed after 48 hr with the B95-8 LCL, which was completely blocked by anti-env serum (Sutkowski et al., 2001), may have confounded analyses of Vβ specificity in some experiments.

Human Endogenous Retroviruses

All humans carry numerous copies of HERV in their genome (reviewed in Lower et al., 1996). As opposed to other retro-elements, HERV contain *env* genes. They are sometimes complete proviruses but they are usually replication defective. There are at least nine families with names like HERV-H, HERV-R, HERV-K, HERV-E, HERV-I, etc., (where the letters denote the specific tRNA primer binding site). HERV-R contains a single gene (ERV-3), but other families are represented by 10-1000 genomic copies. HERV-K, the family of K18, contains 10–50 genomic copies and has significant homology to MMTV. It is a widely studied HERV family because the provirus is complete and potentially capable of producing viral particles, especially in situations favoring viral complementation, as in retroviral infection or gene therapy. Some HERV-K genes are highly expressed in teratocarcinomas and testicular tumors but also at lower levels in placenta and other tissues. HERV-K18 has a unique expression pattern restricted to primary PBL, as determined by a nested RT-PCR assay (Sugimoto et al., 2001). In some studies, *env*-encoded peptides have been detected.

In spite of all these genomic copies of HERV genes, there are no known exogenous equivalents of HERV, as with MMTV or JSRV (Jaagsiekte sheep retrovirus). HERV are thus thought to represent evolutionary fossils from earlier retroviruses. Some of these retroviruses may have plagued apes, as the HERV-K family dates back in evolution to the divergence of Old and New World monkeys.

HERV *env* genes seem to be conserved, suggesting an important biologic function. Beneficial effects of endogenous retroviral *env* expression have been described (Coffin, 1995). Some endogenous *env* confer resistance in mice to MLV, and in chickens to ALV, which are viruses that use the same receptors as the endogenous *env*-encoded protein. This resonates with the role of endogenous MMTV described above. Other possible beneficial effects include mutagenesis due to retroviral insertion. This can functionally delete a gene, but the retroviral LTR can also provide enhancer elements leading to gene activation, as in the case of the duplicated mouse C4 complement gene, or the salivary gland-specific expression of a duplicated human β-amylase gene. Finally, HERV-W *env* genes have been proposed to play a role in placental development by inducing trophoblast fusion to generate the syncytiotrophoblast layer (Mi et al., 2001).

Conclusions and Significance

Are the *sag* of HERV the same as those of MMTV? How do SAg fit into the HERV story, and are all HERV *env* genes endowed with SAg activity? Although these questions remain unanswered at present, we notice some differences between humans and mice. In mice, endogenous SAg cause large deletions of entire Vβ families in certain strains, which are easily detectable at birth. In striking contrast, the human Vβ repertoire is quite uniform at birth in the thymus, blood, and spleen (Doherty et al., 1991; Garderet et al., 1998; Schelonka et al., 1998). There are a few examples of variable Vβ expression, such as Vβ3, Vβ7.2, Vβ13.2, Vβ12.4, Vβ20, Vβ6.1, and Vβ6.7. In each case, genomic differences (genomic deletion, null alleles, or other allelic mutations) in the TCR genes themselves are the cause. One possible conclusion is that endogenous SAg are not expressed in human ontogeny, unlike in mice. Consistent with this conclusion, self-tolerance to some HERV-K *env* products is apparently incomplete. Normal sera contain low levels of specific autoantibodies to HERV-K10 Env synthetic peptides. Higher levels are found after pregnancies and in patients with leukemias and testicular carcinomas (Lower et al., 1996) It is not known whether this also applies to HERV-K18.

Even if HERV-encoded SAg lack biologically significant roles in human fetal development, it is conceivable that they contribute to disease pathogenesis in several different ways. Sutkowski et al. hypothesize that long-term persistent EBV infection in B cells requires T cell help provided by SAg-stimulated T cells. Moreover, they argue that certain human B cell lymphomas require T cell help during oncogenesis. An example of this scenario was provided by elegant studies of B cell lymphomas in SJL mice (Tsiagbe et al., 1993). These tumors express an endogenous MMTV SAg, which elicits T cell help from Vβ16 CD4 T cells, required for tumor cell growth. Another mechanism by which endogenous SAg

can promote tumor growth was discovered in experiments with tumors induced by polyoma virus. Here the endogenous Mtv-7 SAg results in deletion of Vβ6 T cells that are critical in effective antitumor immunity, resulting in an inherited susceptibility to these virus-induced tumors

SAg can either transiently activate T cell subsets or lead to their functional demise. The targeted T cell subset may contain helpful T cells (e.g., those that lead to tumor rejection) or it may include potentially harmful T cells (e.g., those that help the B cell lymphomas in SJL mice). Therefore it is impossible to predict what clinical effects SAg may have. However, it is possible to imagine how SAg may tip the balance one way or another, and this applies not only to tumor immunology but also to infectious diseases and to autoimmunity. Obviously, deletion of a critically necessary TCR specificity could compromise an immune response to an infectious organism. But deletion of a regulatory T cell that downmodulates the response may have the contrary effect. Stimulation of large numbers of T cells as in a SAg response may result in high levels of cytokines. Some microbes use cytokines for their own needs, rather like the tumors in SJL mice. However, *S. aureus* and *S. pyogenes*, the major source of exogenous SAg in humans, are not known to require cytokines for their growth. Indeed, it is a puzzle why these bacteria produce SAg at all. It is also conceivable that viruses, such as HIV, which replicate most efficiently in activated lymphocytes, take advantage of large numbers of SAg-activated T cells (Dobrescu et al., 1995a) and that concurrent chronic infection with EBV, CMV, or KSHV aggravates HIV by this mechanism.

Because of reports of increased Vβ7 cells in the pancreas, spleen, and blood at the onset of IDDM, a pathogenic role for the HERV-K18 has been suggested (Stauffer et al., 2001). The idea that SAg can break tolerance and contribute to autoimmunity is not new (Friedman et al., 1991; Schiffenbauer et al., 1998). As mentioned above, there is inherent incongruity in the responses to SAg: exogenous bacterial SAg can aggravate experimental autoimmune encephalomyelitis (EAE) when given after myelin basic protein (MBP) or inhibit EAE when injected prior to MBP. Different SAg (SEA, SEB) may have opposing effects, indicating that their specificity is important and not just the high levels of cytokines that they provoke. However, the exact mechanisms involved still need to be elucidated. This is important because studies with NOD mice have shown that exogenous SAg (SEA, SEB, and SEC2) administered at 4 or 10 weeks of age either block or ameliorate IDDM. It was further shown that SAg-activated CD4 suppressor T cells could inhibit disease after adoptive transfer (Kawamura et al., 1993). At first glance this seems to contradict the proposed role for the HERV-K18 SAg in human IDDM. However, in addition to the varied specificities of individual SAg, many other factors may play important roles, including dose, timing, and duration of SAg exposure, tissue location of SAg production, and type of APC involved.

It is well recognized that type I interferons (αIFN or βIFN) may influence T cell immunity. This can be beneficial. For example, type I interferons are used clinically for hairy cell leukemia, Kaposi's sarcoma, chronic hepatitis C, and metastatic melanoma. αIFN may also be beneficial in autoimmune diseases like multiple sclerosis, but treatment with type I interferon may sometimes induce autoimmune disorders, especially autoimmune thyroid disease (Belardelli and Gresser, 1996). Whether induction of endogenous *sag* genes, as suggested by Stauffer et al. (2001), plays a role in any of these effects is not yet known.

EBV may not be the only herpesvirus that can transactivate endogenous SAg in humans. Indeed, the original paper on the topic of herpesvirus SAg described a Vβ12-specific SAg activity associated with CMV (Dobrescu et al., 1995b). In part, those studies employed U937 cells exposed to CMV. U937 cells express ERV-3 of the single copy HERV-R family (Larsson et al., 1996). It is currently not known whether this HERV *env* gene is transactivated by CMV or other stimuli. It is also not known whether this *env* gene encodes a protein with SAg-like biological activity. One percent of normal Caucasians lack intact ERV-3 *env* due to homozygosity of an allelic mutation, which results in a premature stop codon (de Parseval and Heidmann, 1998). However, even the shortened version of ERV-3 can encode an ≈25 kDa peptide (de Parseval and Heidmann, 1998). As with the short allele of HERV-K18 (K18.1) (Stauffer et al., 2001), a hypothetical superantigen function could be preserved.

In vivo, the picture may be even more complex. Following reports of SAg-like activity associated with EBV, CMV, and HIV infection, multiple efforts to isolate the putative *sag* from these viruses were unsuccessful. With the discovery of endogenous *sag* genes, the old observations may need to be reexamined. For instance, it is possible that the observed SAg-like activities might have been due to the cumulative effects of multiple viruses (usually present together in vivo) through transactivation of endogenous *sag* genes.

In sum, the potential significance of endogenous SAg is considerable. For instance, can endogenous SAg stimulate CD4 T cells in AIDS patients resulting in increased HIV replication? Can they stimulate select Vβ subsets leading to autoimmunity, like IDDM, as suggested by Conrad and colleagues? Can they delete or anergize critical T cell subsets that would otherwise protect the host from diseases?

One point to remember is that we are constantly exposed to SAg . Most adults have had contact with numerous SAg of *S. aureus* and *S. pyogenes. S. aureus* commonly colonizes the nose of normal persons. As a consequence, we all have serum antibodies to bacterial SAg and these antibodies protect us from toxic shock syndrome. So, how can endogenous SAg make a difference? Some obvious differences are that bacterial SAg exposure is likely repetitive, occurs at low concentrations, and at mucosal and cutaneous surfaces. In contrast, HERV-K SAg expression is apparently induced in professional antigen-presenting cells (B cells), and possibly in virus-infected cells. The exact conditions and mechanisms that are operative in vivo for HERV-K SAg production are not yet fully defined. Perhaps more is involved than just EBV and a little αIFN. Future research will hopefully reveal the secrets of how we manage to sleep with the enemy without getting hurt.

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