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EDITORIAL REVIEW

The potential role of superantigens in inflammatory bowel disease

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INTRODUCTION

Superantigens are the protein products of a number of bacteria and viruses. Their name derives from their ability to stimulate large numbers of T cells compared with that seen with conventional antigens [1].

Among the most widely studied bacterial superantigens have been those derived from *Staphylococcus aureus*. In particular, the enterotoxins SEA, SEB, SEC, SED and SEE responsible for food poisoning and TSST-1 responsible for the toxic shock syndrome have received wide attention. Even though these proteins may share a high degree of sequence homology (SEA and SEE are 92% homologous), they still interact with distinct subsets of T cells [2–5].

Superantigens may also be retrovirally encoded. The protein product of the ORF gene of the mouse mammary tumour virus (MMTV) and the gag gene-encoded p30 antigen of the LP-BM5 variant strain of the murine leukaemia virus are good examples [6–9]. In certain mouse strains, retroviral superantigens may also be produced by endogenous proviral sequences integrated into the genome. A number of MMTVs have been incorporated into the mouse genome and encode the antigens of the minor lymphocyte stimulatory (Mls) system. Over 40 different endogenous MMTV strains have now been identified [10].

Recently, observations of inherited differences in human Vβ2 peripheral T cell levels unrelated to HLA background and *TCRBV2S1* genotype have led workers to suggest that endogenous superantigens may also be present in humans [11].

IMMUNOLOGY OF SUPERANTIGENS

Binding characteristics

Superantigens appear capable of binding to a range of class II MHC molecules including HLA-DR, -DP and -DQ [12], although there may be both isotypic and allelic differences in the efficiency of superantigen presentation between them [13,14]. HLA class II binding appears to be required for the induction of T cell proliferation and cytokine release in the majority of cases, but data derived from MHC class II-deficient macrophage cell lines suggest that several other cell surface molecules are capable of both binding superantigens and triggering cellular activation [13,15]. Unlike conventional antigens, superantigens are not processed by antigen-presenting cells; indeed proteolysis inhibits their superantigen activity [16,17]. Recent crystallographic data confirm that superantigens bind outside the peptide-binding groove [18]. The exact location differs for each superantigen, so that SEB binds to the α1 domain of DR1, whereas mutational studies suggest that SEA binds to the β1 domain, and TSST-1 binds to both α1 and β1 domains [19,20].

SEB's ability to bind multiple DR allotypes may be explained by its exclusive interaction with the DRα chain, but other recent data suggest there may also be multiple binding sites for class II MHC binding on the SEB molecule [21].

The capacity to stimulate large numbers of T cells results from the ability of superantigens to bind with the T cell receptor (TCR) in a Vβ-specific manner [22]. Furthermore, mutations in the TCR's complementarity-determining regions (CDR1–3) generally affect the recognition of conventional MHC-restricted peptide antigens, but not superantigens [23,24]. The TCR region which binds superantigen most strongly is a loop between β-strands D and E of the Vβ, referred to as the fourth hypervariable region (HV4) or CDR4. While the TCR β-chain alone is sufficient for superantigen/class II recognition, there is some evidence to suggest that the TCRα chain and perhaps even the Jβ gene segment may play a role in regulating SA binding specificity [24].

Mediator release

Superantigens can cause the release of a number of cytokines and inflammatory mediators. TSST-1 can stimulate IL-1, IL-2, interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) expression, as can SEB and the mycoplasmal superantigen, MAM. Furthermore, SEB and MAM both induce IL-4 expression and MAM also induces IL-6 expression. SEA induces a similar range of cytokines to SEB, with the exception of IL-1 [2].

In addition, SEB administered orally to monkeys can induce leukotriene release and modify arachadonic acid metabolism [25,26].

Effects on TCR repertoire

There are three reported outcomes of T cell exposure to superantigens: proliferation, anergy, and deletion. These may occur separately or in combination, depending on a variety of factors. MMTV-encoded superantigens may cause intense proliferation of specific Vβ-bearing T cells *in vitro* (a feature which distinguishes Mls responsiveness) or deletion of T cells *in vivo*. Deletion usually occurs during thymic education, when

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characteristic, Vβ-specific gaps in the T cell repertoire occur in both $CD4^+$ and $CD8^+$ cell populations [1,27]. Exposure to a MMTV-encoded superantigen after birth, by suckling, results in almost total thymic deletion of $CD4^+$ and $CD8^+$ T cells, but in the periphery only mature $CD4^+$ T cells are anergized [28]. Treatment of adult mice with exogenous SEB results in initial proliferation followed by either deletion or anergy [29,30].

Human T cells also proliferate *in vitro* in response to superantigen stimulation in a Vβ-specific fashion [31]. Superantigens have also been shown to induce anergy in cultured human T cell lines [32]. *In vivo*, superantigen-driven increases in T cells and TCR-specific mRNA have been observed acutely after exposure to TSST-1 in the toxic shock syndrome and Kawasaki disease (KD) [33–35]. Superantigen-secreting staphylococcal and streptococcal strains have been isolated from KD patients [36], although the reported increases in $V\beta2^+$ and $V\beta8^+$ T cell numbers in this disease have been recently challenged [37]. Increases in cell numbers may persist for several months after the toxic shock syndrome [31,33], but there are no data as to long-term sequelae in terms of T cell responsiveness in either condition.

EFFECTS IN HUMAN GASTROINTESTINAL DISEASES

It has been known for some time that bacterial superantigenic toxins cause acute diarrhoeal illnesses [38]. The systemic symptoms they induce are to a large extent attributable to the massive systemic cytokine release they cause [31].

However, the role that superantigens may play in more chronic conditions such as Crohn's disease (CD) has been highlighted ever since the discovery that $V\beta8^+$ T cells were elevated in the peripheral blood and mesenteric lymph nodes of a subset of CD patients compared with controls [39]. This study demonstrated that CD patients, even within the same family, could have either elevated or normal levels of $V\beta8^+$ T cells in their peripheral blood. Furthermore, neither HLA genotyping nor *Bam*H1 restriction fragment length polymorphism (RFLP) of Vβ8.1 predicted which CD patients would have elevated $V\beta8^+$ T cell levels, suggesting that external rather than inherited factors were responsible for the changes in T cell repertoire [39]. Further studies by the same group demonstrated that intestinal epithelial cells (IEC) were capable of presenting superantigens in about 50% of normal individuals. Those IEC not capable of presenting superantigens were refractory to treatment by accessory cytokine addition, the induction of MHC class II expression and the suppression of prostaglandin E_2 production [40]. This group also quoted preliminary data suggesting that IEC from uninvolved areas of bowel in CD patients also showed variability in their ability to present superantigens, perhaps explaining why only a subset of CD patients exhibited changes in their $V\beta8^+$ T cell repertoire [40].

In a recent issue of this journal, elegant experiments using TCR-directed cytotoxicity have extended these studies further by demonstrating that the peripheral blood $V\beta8^+$ T cell population is functionally abnormal in some CD patients [41]. Cultured $V\beta8^+$ T cells from these patients showed decreased cytotoxic activity compared with controls, despite equivalence in total or $CD8^+$ $V\beta8^+$ T cell numbers. Furthermore, although the $V\beta8^+$ T cells from CD patients proliferated in response to treatment with the staphylococcal enterotoxin E superantigen (SEE), neither SEE nor an anti-Vβ8 MoAb could induce increased cytolytic activity from this T cell subset compared with controls.

However, this is not the only reported defect within human $V\beta8^+$ T cells. A recent paper examining $V\beta8^+$ T cell responsiveness in HIV^+ individuals reported a lack of proliferation and IL-2R expression in these T cells after stimulation with either an anti-Vβ8 MoAb or the $V\beta8^+$ T cell-stimulating superantigens, SEE and ETA [42]. This defect could not be reversed by the addition of exogenous IL-2 or IL-4.

It is already known that superantigen exposure *in vitro* can inhibit T cell proliferation without impairing cytotoxic function [43,44], and can also abrogate antigen responsiveness, leaving proliferative capacity unaltered [32]. While the second paper did not examine the cytotoxic capacity of the $V\beta8^+$ T cells in HIV^+ individuals, these studies would appear to be an *in vivo* corollary demonstrating that proliferation and antigen responsiveness are two separate functional characteristics capable of being inhibited separately.

While these findings are indeed exciting, a certain amount of caution must be exercised. Proliferative and cytotoxic anergy may have been observed *in vitro* after superantigen exposure, but the demonstration of cytotoxic and proliferative anergy *in vivo* is not, in itself, evidence of such exposure. In human diseases where superantigens have been demonstrated, such as toxic shock syndrome and KD, initial T cell proliferation and TCR mRNA up-regulation have been observed, but the long term sequelae in terms of T cell function are not known [33–36]. Until such data are known, the role of superantigens in the pathogenesis of a number of conditions, including inflammatory bowel disease, can only be surmised.

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