BACTERIAL SUPERANTIGENS AND ORGANISM PHYSIOLOGICAL STATUS: A THEORETICAL APPROACH

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Abstract: The present review was undertaken in order to present the bacterial superantigens influences on the organism physiological status. In recent years, a great deal of a new knowledge about a group of microbial proteins known as superantigens has been generated. These molecules have elicited tremendous interest because they interact with the immune system in a nonconventional manner and can potentially trigger diseases such as toxic shock, food poisoning, and autoimmunity. Moreover, evidence from clinical observations, animal models of disease, and studies with cell lines indicate an intimate association between bacteria (and bacterial products) and the pathophysiology of many nervous disorders. In summary, knowing the immunological and biological effects of superantigens will help to discern the mechanism of pathogenesis of a number of diseases linked to microbial infections and will offer new possibilities for the development of novel and effective therapeutic strategies.

INTRODUCTION

In international scientific world, the study of the superantigens (SAg) effects have received considerable attention in terms of their strong T cell stimulating effects and the promotion of pathophysiological conditions, including septic shock and autoimmune disease (Llewelyn and Cohen, 2002). The best studied of these SAg are the enterotoxins produced by the gram positive bacteria *Staphylococcus aureus*, and in particular, the staphylococcal enterotoxins A and B (SEA/SEB) (Llewelyn and Cohen, 2002). As T cell stimuli, SEA and SEB are unique in that T cell activation proceeds independently of intracellular digestion by antigen processing cells (APC), albeit still involving MHC Class II molecules (Yagi et al., 1991). Ultimately, however, T cell activation occurs via the T cell receptor (TCR) (Hong et al., 1996). In animal studies involving challenge with SEA or SEB, this interaction has been shown to result in rapid cytokine gene induction, and appearance of measurable levels of plasma TNF and IL-2 within 1–2 h of administration (Sundstedt et al., 1994). Expanding on the initial observations by Gonzalo et al. (1993) of increased corticosterone production following SEA or SEB treatment, it has been firmly established that consequent to administration of these bacterial superantigens to mice and rats, there are significant alterations in endocrine, neurobiological, and behavioral functions (Kawashima and Kusnecov, 2002; Rossi-George et al., 2004). For instance, challenge of BALB/cByJ mice with SEB stimulates the HPA axis, resulting in increased plasma levels of ACTH and corticosterone (Kusnecov et al., 1999). The neuroendocrine and behavioral effects of SEA and SEB may be dependent on the specificity of each particular bacterial SAg for their target TCR (Gonzalo et al., 1993). Indeed, among murine T cells, the variable region on the b-chain of the TCR is encoded by at least 20 different genes that can give rise to as many unique amino acid sequences (Gonzalez-Quintial et al., 1995). This allows for further differentiation of CD4 and CD8 T cells into subtypes based on the particular Vβ gene contributing to the construction of the TCR. Staphylococcal enteroxins A and B possess differing affinities for these Vβ subtypes, with SEB preferentially stimulating Vβ8 T cells, while SEA has high affinity for Vβ3 and Vβ11 T cells (Gonzalo et al., 1993). Moreover, irrespective of the mouse strain used, the neuroendocrine effects of both SEA and SEB require the presence of functional T cells, as confirmed using either cyclosporine A immunosuppression or RAG-1 knockout mice, which lack functional T lymphocytes (Kawashima and Kusnecov, 2002). The behavioral effects of SEB have been confined to the study of ingestive behavior (Kusnecov et al., 1999). Challenge with SEB results in a significant reduction in the consumption of a novel solution, an effect that is maintained by the novelty of the context in which the solution is presented (Kusnecov et al., 1999). Since exposure of naive animals to novel contextual or gustatory stimuli activates the HPA axis and increases latency to interact with the novel stimulus (Kopp et al., 1999), the inhibitory effects of SEB on novel food ingestion may represent an augmentation of anxiety-like processes. Lypopolysaccharide (LPS) from *Escherichia coli*, also known as endotoxin, is a molecule on the outer portion of gram-negative bacteria. LPS has been the agent of choice of many neuronal immune studies due to the ability of LPS to reliably induce a rapid host response in a dose dependent manner. Some of the most readily-activated components of the LPS-induced homeostatic response are cytokines (TNF, IL-1, IL-6), which injected in their pure form, mimic many effects of LPS on the host (Abbas el al., 1997). Previous studies have reported the effects of LPS on the animal organism functions. For instance, neonatal exposure to bacterial products (LPS) in rats influences reactivity to stress, immune regulation, and susceptibility to disease (Boisse et al., 2004; Hritcu et al., 2006; Hritcu and Stefan, 2009). Behavioral alterations in particular may be the result of damage by cytokines during development to the cortex and especially the hippocampus, a brain region important to the pathology of behavioral and cognitive disorders (Bauman et al., 1997). Cytokine receptors are distributed throughout the brain, with high densities in the hippocampus (Cunningham and De Souza, 1993). For this reason, the

hippocampus is thought to be particularly vulnerable to immune-related alterations (Lynch el al., 2004). The neonatal exposure to bacterial Escherichia coli in rats is associated with memory impairment in adulthood (Bilbo et al., 2005). Remarkably, however, this impairment is only observed if an immune challenge (LPS) is administrated immediately after learning experience.

The increase of the information volume regarding the relationships which appear between bacterial superantigens and animal organism was possible by using some modern investigation methods. Conventionally, molecular sieving and ion exchange chromatography have been used either independently or in tandem for purification of staphylococcal enterotoxins (Dainiak et al. 2005). Isoelectric focusing has been proved useful for purification of small amounts of staphylococcal enterotoxins but on a preparative scale the procedure has generally been used in combination with ion exchange chromatography and gel filtration.

MECHANISM OF ACTION

SAgs are characterized by their ability to bind both MHC class II molecules and T cell receptors (Seth et al., 1994). This occurs in a sequential fashion. The sole purpose of SAgs appears to be to bring these two critical molecules together in order to activate as many T cells as possible. The net result is the release of a large and sudden bolus of cytokines which causes the acute condition toxic shock (Fast et al., 1989). The histocompatibility class II molecule, despite its polymorphism, is the principal cell receptor for all SAgs but the affinity for MHC class II varies depending on the class II molecule and the SAg (Norrby-Teglund et al., 2002). All SAgs examined so far display higher affinities towards human MHC class II molecules than mouse class II, which explains partly why SAgs are several orders of magnitude more potent on human T cells than mouse T cells. A variety of binding modes exist to both MHC class II and TCR which indicates the lengths that the bacteria have gone to target these two critical molecules of the adaptive immune response.

STAPHYLOCOCCAL EXOTOXINS AS SUPERANTIGENS

A number of gram-negative and gram-positive bacteria have been shown to produce or suspected of producing superantigens. Among the best characterized superantigens are the family of pyrogenic exotoxins produced by *Staphylococcus aureus* and *Streptococcus pyogenes* (Bergdoll et al., 1981; Schlievert, 1993). The known staphylococcal pyrogenic superantigens include the staphylococcal enterotoxins (SEs) A through E (SEA through SEE), toxic shock syndrome toxin-1 (TSST-1), and exfoliative toxin (Schlievert, 1993). In *S. pyogenes*, also known as group A streptococci, the streptococcal pyrogenic exotoxins (SPe) represent a family of superantigens which includes SPeA, SPeB, SPeC, SPeF, and SSA (Tomai et al., 1992).

LIPOPOLYSACCHARIDE AS SUPERANTIGENS

Because work with replicating pathogens such as viruses or bacteria could be potentially dangerous for experimental subjects and could introduce extra variability into an experiment, many experiments that involve proinflammatory cytokine production use lipopolysaccharide (LPS) as an alternative. LPS is a ubiquitous component of the cell wall of Gram-negative bacteria, and triggers an immune response through the toll-like receptor-4 complex (Pasare and Medzhitov, 2004). Once bound, immune cells respond to LPS by releasing proinflammatory cytokines (Dantzer, 2004). Acute and chronic administration of LPS leads to “sickness behavior” which consists of decreased social exploration, anhedonia, decreased sexual behavior, decreased feeding behavior, and decreased locomotor activity (Borowski et al., 1998; Lockey et al., 2009). These changes in an organism's behavior are not inflexible changes; rather, they represent a

change in motivational state that is adapted to the needs of the current situation, to aid in recovery from illness (Dantzer, 2004).

IMMUNE INFLUENCES ON LEARNING AND MEMORY

One of the best examples of the interplay between the brain and the immune system is the conditioning of certain aspects of the immune response to LPS through the conditioned taste aversion (CTA) paradigm. Typically, CTA involves exposure to a novel flavored solution, after which the animal is injected with some noxious substance, and on subsequent exposures the animal avoids the solution. CTA is an example of “one trial learning” and produces very stable and prolonged conditioning. Several symptoms or features of the acute phase response to LPS can be conditioned by CTA, such as fever, sleep alterations (Bull et al., 1994), plasma iron concentrations (Exton et al., 1995), and anorexia (Exton et al., 1995). In addition to the conditioning of immune responses, the immune system can also have deleterious effects on learning and memory.

The effects of LPS on the CNS typically develop two to four hours after exposure and can last as long as 24 hours. During this time, the release of cytokines can produce noticeable deficits in learning and memory. Administration of LPS (or cytokines) negatively affects performance on a variety of behavioral paradigms, such as two-way active avoidance, Morris water maze, and autoshaping, among others (Sparkman et al., 2005). Such cognitive effects are not surprising, because of the high density of IL-1 receptors in the hippocampus, is a brain structure that is widely known for its role in learning and memory (Schneider et al., 1998). Pugh et al. (1998) demonstrated that LPS administration impairs memory consolidation in contextual (hippocampus dependant), but not auditory fear conditioning (not hippocampus dependent). The same deficits were also seen with central administration of IL-1β, and the effects appear to be specific to tasks that depend on the hippocampus, such as contextual fear conditioning (Rachal-Pugh et al., 2001). Barrientos et al. (2002) further demonstrated that IL-1β specifically blocks learning of the context, and that latent exposure to the context prior to IL-1β administration can ameliorate these effects.

In addition to memory consolidation deficits, exposure to cytokines can impair memory acquisition as well. In an autoshaping task, animals injected with LPS during acquisition of a lever-pressing task showed less lever pressing than control animals, indicating that these animals failed to associate the lever with the administration of a food pellet.

Clearly immune activation can influence the CNS and lead to cognitive impairments in various hippocampus-dependent testing paradigms, including the Morris water maze (Kohman et al., 2007), autoshaping, contextual fear conditioning (Pugh et al., 1998), and two-way active avoidance conditioning (partially hippocampus-dependent). Because many individuals suffer from the cognitive effects of long-term inflammatory conditions or treatment with cytokine-based therapies, further research examining the potential mechanisms or means of attenuating these effects is warranted.

SUPERANTIGENS IN HUMAN DISEASE

Food poisoning

The staphylococcal superantigens SEA-SEE and SEG-SEI are potent gastrointestinal toxins responsible for staphylococcal food poisoning. Quantities of less than 1 mg of toxin are

sufficient to trigger vomiting in humans. This enterotoxin function appears to be distinct from the SAg activity but this remains controversial. A highly flexible disulphide-loop within the N-terminal domain has been implicated with the emetic properties, but the exact mechanism that leads to the disease or a specific receptor molecule have not yet been identified (Alber et al., 1990).

Toxic shock syndrome (TSS)

Classical toxic shock syndrome (TSS) caused by *S. aureus* can be considered as a capillary leak syndrome and includes symptoms, such as hypotension, rash, desquamation, fever and major organ involvement. Like endotoxic shock, TSS is mediated through TNF-a. TSST is regarded as the primary causative agent for menstrual TSS, which is associated with the use of certain tampons, particularly those of high absorbency that promotes the growth of *S. aureus*. In contrast to other staphylococcal SAgs, TSST has the ability to cross the mucosa. TSST and other staphylococcal Sags have been associated with non-menstrual TSS, which can occur in any patient population (Dinges et al., 2000). This is supported by the observation that these toxins induce TSS-like symptoms in animal models in the rabbit and in rodents (Bonventre et al., 1993).

Streptococcal toxic shock syndrome (STSS)

STSS, caused by *S. pyogenes*, is the most severe form of invasive streptococcal disease, with mortality rates of up to 50%. The clinical symptoms are very similar to those in TSS, but STSS is often associated with bacteraemia, myositis or necrotizing fasciitis (Stevens, 2000). Streptococcal SAgs have been implicated in STSS and supporting evidence includes the following. The *spe-a* and *spe-c* genes were found at higher frequencies in isolates from STSS patients compared to control groups (Musser et al., 1991), lack of protective anti-SAg antibodies was found to be associated with an increased risk for STSS (Basma et al., 1999) and circulating SAgs were found in several patients suffering from STSS (Sriskandan et al., 1996).

CONCLUSIONS

Superantigens are a fascinating group of molecules that have captured the attention of scientists from many different fields. Understanding their mode of action and structure-function relation will help reveal the underlying mechanisms in a number of diseases that have remained a mystery for years. It is important to realize that superantigens differ in their in vitro and in vivo effects on the immune system and that their biological activity may depend to a great extent on the host and environmental factors (e.g., coincidental viral infection, stress, or hormonal changes) that may potentiate its interaction with the host.

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