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Toxicology elsewhere

The purpose of this section in *Human and Experimental Toxicology* is to draw attention to papers that are of significance to toxicologists but may be missed because, in the main, they have not been published in mainstream toxicology journals. In addition the papers selected attempt to reflect topics of the liveliest current research interest as well as those which impinge upon the professional practice of toxicology. In order to assist the assimilation of such new material an expert commentary is provided and because these aim to focus succinctly on the advance made they should also serve as mini-reviews to keep us all abreast of our rapidly developing, multi-disciplinary, subject.

Adding insult to injury

Cross talk between the immune system and the nervous system in response to injury: implications for regeneration

Lotan M and Schwartz M
FASEB J, 1994; **8**: 1026-1033

Abstract

The central nervous system has long been regarded as an immunologically privileged site. Accumulating evidence suggests, however, that the privilege is not total, and that certain immune functions involving immune components and resident glial cells can operate in the central nervous system. The nervous and immune systems interact during normal development, but in the mature brain their interaction is restricted mainly to cases of pathogenic infections and traumatic lesions. The focus of this review is on bidirectional interactions between immune and neuroglial components in response to nerve injury. The macrophage is the most ubiquitous of the immune-derived cell types associated with injury. Its role, as in any other organ, is tissue remodelling and promotion of healing. Macrophage activities include removal of dead tissue and debris by phagocytosis, lipid recycling, and secretion of a wide spectrum of cytokine possessing trophic, mitogenic, and chemotactic properties. These activities affect the behaviour of resident cells in the vicinity of the wound. We discuss the possible association of these cytokines with the ability of injured nerves to regenerate. Finally, we consider the apparently conflicting effects of post-traumatic inflammation on the recovery of function.

Comment

This timely review highlights the current controversy regarding inflammatory responses in the central nervous system. Are they the driving force behind repair of the damaged organ or do they represent a reaction which can cause damage to neural elements. For those interested in stimulating repair and limiting damage, inflammation poses a dilemma, should it be prevented or encouraged.

The endogenous cells of the CNS involved in inflammatory responses are microglia and astrocytes. In the normal brain, molecules associated with inflammatory responsiveness are down regulated in microglia compared to their counterparts in other organs of the body. Under normal conditions perivascular microglia and those associated with the CSF compartment turn-over, while those within the parenchyma of the brain show very limited turn-over. The CNS macrophage population can be augmented by recruitment of cells from the circulation in pathological states.

Although macrophages are the major source of cytokines at sites of injury, reactive astrocytes also serve as a primary source of multiple growth factors and cytokines. Many cytokines produced by macrophages can also be produced and secreted by astrocytes (e.g. IL-1, IL-6, TNF. TGF-β, bFGF, PDGF). Moreover, astrocytes also produce cytokines which can act on macrophages, such as colony stimulating factors (CSFs). In addition reactive astrocytes produce prostaglandin and lipocortin. Astrocytes and macrophages therefore produce factors which regulate each other. Both cell types also produce and secrete molecules which in tissue culture can kill the non dividing cells of the brain, neurones and oligodendrocytes.

With the exception of T-cell mediated inflammatory responses it is difficult to induce acute inflammatory responses in the CNS. Thus, injection of bacterial lipopolysaccharide, IL-8, or TNF-α, which would elicit a marked polymorphonuclear leukocyte response in peripheral tissue has no similar effect in the CNS. When polymorphonuclear leukocytes do enter damaged tissue, such as in infarcts, they do so in small numbers and macrophage influx is slow compared to that seen in the periphery. So what initiates macrophage influx? At one time breakdown of the blood brain barrier was thought to be a major factor, however, there is now good evidence that this is not a requirement for monocyte recruitment. A possible source of cytokines to induce the initial reaction of microglia and astrocytes are damaged neurones, which could release trophins, such as TGF-βs, acidic and basic FGF and

possibly M-CSF into wound sites to stimulate the initial response of microglia and astrocytes.

While the control of inflammatory responses within the CNS has the beneficial effects of preserving function and protecting vulnerable post mitotic cells from the non-specific damaging products of inflammatory cells it appears to have consequences for regenerative responses which depend on, or are influenced by, the cytokines and growth factors liberated by cells involved in the inflammatory process. Axon regeneration in the PNS and its absence in the CNS has long been linked to the failure of the CNS to initiate inflammatory responses comparable to those in the PNS and this review gives a clear account of experiments designed to probe this issue.

As yet no firm conclusions can be drawn concerning the balance between protection and potential toxic effects of inflammation in the CNS, however the readers attention is drawn to the complexity of the issue and the need to separate the immediate post injury situation where inflammation may be harmful especially when the normal protective cells of the CNS, the astrocytes, may be compromised and the later times when the cytokines and growth factors secreted by responding cells may have a beneficial effect for attempts at regeneration. What is not addressed is the fact that this latter phase will be influenced by the inherent properties of the CNS which inhibit inflammation. It is the nature of these we know so little about.

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Virtual reality

Spectrum of *in vivo* hprt mutations in T lymphocytes from atomic bomb survivors. I. Sequence alterations in cDNA

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Abstract

Recently, we found an elevated frequency of 6-thioguanine-resistant (TG^r) mutations at the hyoxanthine-guanine phosphoribosyltransferase (*hprt*) gene in T cells of peripheral blood from atomic bomb survivors and slight, but significant, positive correlation between the frequency of mutation and radiation dose. Southern blot analysis of DNA from TG^r mutant T cells of atomic bomb survivors, however, failed to show a significant difference between the control and survivor groups. We here report mutational events at the *hprt* locus of TG^r mutant T cell clones from atomic bomb survivors as found by (i) the multiplex polymerase chain reaction (PCR) and (ii) the reverse transcription (RT)-PCR of cDNA and sequencing. The numbers of independent TG^r mutant T cell clones examined were 41 from a control group of 18 individuals who had received less than 0.005 Gy and 50 from an exposed group of 24 individuals who had received more than 1.5 Gy (mean dose 2.45 ± 0.85 Gy). Gross structural alterations, which were detected by multiplex PCR as a loss of or shift in *hprt* exon-containing fragments of genomic DNA, were found in 10–15% of the clones from both groups, thus indicating that there was no significant difference between them. The altered sequences in the HPRT cDNAs recovered from both groups were of various types. Similar proportions of base substitutions (~45%), deletions or insertions (~25%) and exon skipping (~20%) were found in both groups, indicating that neither the gross structural alterations in the genomic DNA nor sequence alterations in the *hprt* cDNA of both groups differ significantly. These results confirm our previous observation that A-bomb-induced $HPRT^-$ mutant cells have mostly been eliminated from the peripheral blood over the decades that have elapsed since exposure. Some unique features of the mutational sequence alterations found are also described.

Comment

For 25 years, the *hprt* gene has been the most widely used selectable marker for the detection of gene mutation in rodent and human cells, and has been used extensively in genetic toxicology. From a mechanistic point of view, the value of these studies has been considerable enhanced by an ability to determine the spectrum of mutations detected at this locus. Initially this was undertaken by Southern analysis, but the advent of PCR enabled mutation spectra to be determined in a variety of cultured human and rodent cell lines, in addition to germ-line mutations in Lesch-Nyhan (hprt) patients and somatic mutations in circulating T-cells of normal and DNA repair-deficient humans. An admirable database of this extensive literature has been compiled and maintained by Cariello and Skopek.[1,2] Both animal studies *in vivo* and *in vitro* cell culture experiments, where analysis of true 'spontaneous' or 'induced' mutants is possible, have provided highly informative data.[3,4] By applying these techniques to human population monitoring, it was anticipated that spectrum analysis might enable the 'footprint' of exposure to specific mutagen (or mutagen classes) to be detected,[5] as has been suggested in the case of *hprt* mutations and ethylene oxide exposure,[6] or *p53* mutations and vinyl chloride exposure.[7]