neurons of a rat conditioned to associate this tone with footshocks. Thus, the neuronal basis of fear conditioning has begun to be unravelled (McKernan and Shinnick-Gallagher 1998; Rogan *et al* 1998).

While a tone-footshock association may be made following a single pairing of the two stimuli, for the same tone to be associated with a reward, such as a food reward, several pairings of the two stimuli are usually required. Thus, fear-based associations are learned faster than reward-based associations. When one considers the evolution of mammals, it is easily apparent why brains may function in this manner. The apparent efficiency of fear-based training compared to the reward-based method may superficially suggest alterations to the now-fashionable, politically-correct approach to rearing children or students. Perhaps the traditionalists had it right: "spare the rod and spoil the child". However, fear-based learning, while efficient, is extremely limited. The emotion of fear appears so strong that, in the fearful state, new associations are actively blocked. This blocking may occur via the secretion of opiates by amygdalar neurons which prevent the formation of new associations (reviewed by Fanselow 1998). In evolutionary terms, our brains may have evolved to ensure that fear (such as that generated by the sight of a hungry tiger) causes us to ignore all non-fearful stimuli until the source of fear is gone. Thus, fear-based training programs, while efficient, probably have a price that should greatly restrict their general use.

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CpG-containing oligodeoxynucleotides as new generation adjuvants in DNA and protein vaccines

Since ages, Freund's Complete Adjuvant (FCA) has been used by immunologists to boost the immune response in experimental animals. Due to inflammatory side effects however, use of FCA is not permitted in humans and search is on for a better adjuvant. Recently several reports have shown that bacterial DNA acts as potent adjuvant, promoting a T-helper 1 (Th1) immune response. An immune response can be classified as a Th1 type characterized by the generation of cytokines like interferon gamma (IFN-γ) and interleukin-2 (IL-2), or a Th2 type in which cytokines like IL-4, IL-5, IL-10 and IL-13 are produced. Th1 cytokines influence the immune response and push it towards the generation of cell mediated immune (CMI) responses like cytotoxic T cells (CTL)

and DTH responses, as well as a switch over of immunoglobulin isotype to IgG2a. Th2 cytokines tilt the immune response towards production of other isotypes of immunoglobulins and block CMI responses. In many disease conditions, one of the two (Th1 or Th2) responses may impart protection while the other type may not be useful or even enhance the susceptibility of the host to the infection. For instance, in tuberculosis and AIDS, a Th1 immune response appears to be protective. Individuals infected with HIV may remain healthy as long as they have a Th1 type of immune response to the viral antigens. Symptoms of AIDS appear in these persons as their immune response shifts from a Th1 to a Th2 type (Clerici and Shearer 1993). The reason for this shift is not known. In this context, it is interesting that the use of bacterial DNA as adjuvant specifically promotes a Th1 response. These indications came as a spin off from the work being done on DNA vaccines where the gene encoding a given immunogenic protein is incorporated in a suitable vector and the modified vector is used as vaccine (Robinson and Torres 1997). In these experiments, administration of vector alone, as control, also resulted in potentiation of the immune response (Leclerc *et al* 1997). The effect was tracked down to the presence of CpG sequences in bacterial DNA. CpG sequences are also present in mammalian DNA but are heavily methylated and therefore not effective. Insect DNAs which have non-methylated CpG sequences are also potent adjuvants (Sun *et al* 1998). Synthetic oligodeoxynucleotides containing CpG sequences (CpG ODN) have been shown to be effective adjuvants when administered along with an antigen and tilt the immune response towards a Th1 type (Chu *et al* 1997). Davis *et al* (1998) recently demonstrated that immunization of mice with Hepatitis B surface antigen along with CpG ODN resulted in a strong Th1 response (antibody of IgG2a isotype and CTLs) but use of alum adjuvant with the same antigen resulted in a Th2 response (antibodies of IgG1 type and no CTLs). These results may mark a milestone in the evolution of vaccines (Krieg *et al* 1998). CpG sequences may either be incorporated into DNA vaccines or CpG ODNs mixed with protein vaccines to not only boost the immune response, but also promote a Th1 kind of response. The mechanism of action of CpG sequences in generating a Th1 response is not well understood. CpG containing bacterial DNA is known to induce the secretion of TNF and IL-12 by macrophages (Sparwasser *et al* 1997). NK cells generate IFN-γ in response to IL-12. As IFN-γ is known to inhibit a Th2 immune response, its early release may set the stage for a robust Th1 immune response.

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