Th1-type cytokine mRNA in rheumatoid arthritis mononuclear cells induced by streptococcal pyrogenic exotoxin A

Sir, Subpopulations of helper T lymphocytes produce different cytokines, such that Th1 cell products characteristically include IFN-γ and IL-2, whereas Th2 cells produce IL-4 among others (reviewed in [1, 2]). Studies in rodent models suggest that autoimmune diseases can be promoted by Th1 cytokines and downregulated by Th2 cytokines. Although the validity of the Th1/Th2 paradigm is influenced by many factors, Th1 cytokines are nevertheless linked with pathogenicity in RA [3] and other autoimmune conditions.
The family of bacterial exotoxins known as superantigens is implicated in autoimmune pathogenesis, but the role of superantigens in RA remains contentious. No consistent evidence for selective TCR V gene family usage by T cells in RA joints has emerged (reviewed in [4]), but the interpretation of such findings is debatable since T cells activated by superantigens may be subsequently deleted from the repertoire [5]. We have investigated an alternative possibility, namely, that bacterial superantigens may promote RA by differentially modulating Th1/Th2 cytokine production.

Streptococcus pyogenes, which induces autoimmune sequelae, produces several superantigens including pyrogenic exotoxin A (SPEA) [6, 7]. We compared recombinant SPEA (rSPEA) with phorbol 12-myristate 13-acetate (PMA) + phytohaemagglutinin (PHA) for inducing Th1 (IFN-γ, IL-2) and Th2 (IL-4) mRNA expression in RA peripheral blood mononuclear cells (PBMC). Levels of mRNA expression, rather than cytokine production, were assayed because of their sensitivity to differential effects of stimulus type or disease status. PBMC were obtained from 14 RA out-patients (12 females aged 40–77 yr, two males aged 40 and 68 yr) and nine healthy donors. PBMC at 4 × 10^6/well in 24 well plates were stimulated with either rSPEA 100 nm or PMA 10 ng/ml + PHA 10 μg/ml. Four hours later, total cellular RNA was extracted. A modified competitive PCR method was used to measure IL-2, IFN-γ, IL-4 and β2-microglobulin mRNA expression [8]. The technique involved co-amplification of cellular RNA-derived cDNA with a multispecific synthetic cDNA added as an internal standard, using primer pairs common to both templates. The internal standards have the same sequence as the cellular template, except for either an internal mutated restriction site or a small intron, so the distance between standard RNA differs from that in the respective cellular RNA. The primer pair was added, and both standard and cell cDNA co-amplified by PCR. The standard and cell amplicon were separated by agarose gel electrophoresis and each band quantitated by densitometry using ImageQuant software. Amounts of cytokine mRNA were expressed by the ratio [OD of cell amplicon]/[OD of standard amplicon]. Wilcoxon’s rank sum test was used for statistical analysis.

There was no cytokine mRNA expression in fresh PBMC or in PBMC cultured for 4 h in medium alone. In healthy PBMC, mean levels of mRNA for IFN-γ were higher than for IL-4 irrespective of stimulus type. In RA PBMC stimulated with PMA + PHA, IFN-γ mRNA was significantly lower (P < 0.05) compared with healthy PBMC. However, IFN-γ (P < 0.01) and IL-2 (P < 0.001) were significantly higher in RA PBMC stimulated with rSPEA compared with stimulation by PMA + PHA. In contrast, there was no difference in IL-4 induced in RA PBMC when stimulated with PMA + PHA compared with rSPEA. There was no effect of disease status or stimulus type on β2-microglobulin mRNA, confirming that equal amounts of total RNA were present in each analysis (Fig. 1).

Therefore, although RA Th1 responses to PHA + PMA stimulation were significantly reduced compared with healthy donors, this apparent deficit was overcome by rSPEA which stimulated comparatively high levels of both Th1 cytokines. This cannot be ascribed to a putatively higher frequency of SPEA-responsive T cells in RA compared with healthy PBMC because there was no difference between the amounts of IL-2, IFN-γ or IL-4 mRNA in SPEA-activated RA compared with healthy PBMC, and also because we found no differences between responses by SPEA-activated RA and healthy PBMC in proliferation assays (unpublished data). Our interpretation of these data is based on the fact that PHA + PMA stimulation induced significantly lower amounts of Th1 mRNA in RA compared with healthy PBMC. Similarly, others have shown reduced IFN-γ, but not IL-4, production by RA T cells stimulated with anti-CD3 + anti-CD28 monoclonal antibodies [9, 10], indicating that Th1 downregulation also occurs using stimuli which mimic physiological T-cell activation more closely. The biological significance of downregulated Th1 induction in RA is unknown. However, our finding that SPEA did not induce this effect raises the possibility that SPEA might induce Th1 cytokine production in RA despite the existence of putative protective regulatory mechanisms aimed at Th1 reduction. The extent to which this might also apply to other bacterial superantigens remains to be determined.

Most previous theories of superantigen involvement in autoimmune pathogenesis have proposed breaking tolerance by activation of self-reactive T cells. Instead, we suggest that the propensity of superantigens for differential egress by T cells in RA compared with healthy PBMC or IL-2, IFN-γ, IL-4 and β2-microglobulin mRNA expression [8]. The technique involved co-amplification of cellular RNA-derived cDNA with a multispecific synthetic cDNA added as an internal standard, using primer pairs common to both templates. The internal standards have the same sequence as the cellular template, except for either an internal mutated restriction site or a small intron, so the distance between standard RNA differs from that in the respective cellular RNA. The primer pair was added, and both standard and cell cDNA co-amplified by PCR. The standard and cell amplicon were separated by agarose gel electrophoresis and each band quantitated by densitometry using ImageQuant software. Amounts of cytokine mRNA were expressed by the ratio [OD of cell amplicon]/[OD of standard amplicon]. Wilcoxon’s rank sum test was used for statistical analysis.

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Most previous theories of superantigen involvement in autoimmune pathogenesis have proposed breaking tolerance by activation of self-reactive T cells. Instead, we suggest that the propensity of superantigens for stimulating Th1-type cytokine production, irrespective of T-cell antigen specificity, may be more relevant to their autoimmune pathogenic potential.

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Fig. 1. Mean and s.e. mRNA expression by RA and control PBMC following stimulation with either rSPEA or PMA + PHA. (a) IFN-γ. The asterisk denotes a significant difference between PHA/PMA and rSPEA stimulation in RA patients ($P < 0.01$). (b) IL-2. The asterisk denotes a significant difference between PHA/PMA and rSPEA stimulation in RA patients ($P < 0.001$). (c) IL-4. (d) β2-microglobulin.

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