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The results show that AUG rats immunized with autologous thyroglobulin produce polyclonal autoantibodies against thyroglobulin and the rise in antibody titre correlates strongly with thyroid destruction. This animal model of autoimmune thyroiditis resembles the human disease in many respects and the IEF spectrotypes show that polyclonal activation of B-lymphocytes is involved in most animals, as seen in the majority of patients with Hashimoto’s disease (Stott et al., 1986). Sequential studies on individual animals revealed quantitative and qualitative changes in spectrotypes. An oligo-/polyclonal spectrotype appeared from the earliest expression of individual clones, most of the bands declining together as the response faded. Thus, most of the B-lymphoclastotypes in the region pH 7.5–8.2 which appeared at week PI 8.3 and type that persisted for 8 weeks. Rat D had three dominant clonotypes in the region pH 7.5–8.2 which appeared at week 1, fading away at week 8. A new clonotype (the two bands at pH 8.3 and 8.4, arrows) appeared at week 2.

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clonotype appeared at weeks 15–16 (tracks B2 and C3) and then died away. Rats D and E had biclonal spectrotypes at weeks 12 and 16, respectively (tracks D3 and E4). The acidic clonotype of rat E was replaced by a new one at 27 weeks (track E6). Similar changes occurred in the spectrotyp e of rat F. A dominant group of basic bands appearing at 20 weeks (track F5) to be replaced in turn by a clonotype of four bands (arrows, tracks 6 and 7).

Thymectomy followed by sublethal irradiation was shown to induce autoimmune thyroiditis in rats by Penhale et al. (1975). Lymphocytic infiltration of the thyroid and production of anti-thyroglobulin lead to destruction of thyroid follicles with a more delayed time course compared with the immunized AUG rat model. IEF spectrotypes of anti-thyroglobulin autoantibodies were much more restricted in the clonal antibody.

Some clonotypes persisted over the period of the study, giving a maximum lifespan for an autoantibody secreting B-cell clone of at least 16 weeks.

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Impairment of erythrocyte–antibody rosette formation by human lipocortin 1 on human leucocytes

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Lipocortins, a family of glucocorticoid-induced proteins of Mr 33000–38000, inhibit phospholipase A2 activity in vitro and have anti-inflammatory effects in animal models of inflammation. In view of a reported intra-membrane association between phospholipase A2 and Fcγ receptors on human leukaemic B-cell lines (Suzuki et al., 1980), we have examined the effect of lipocortin 1 binding to human mononuclear cells and neutrophils on the expression of Fcγ receptors. Human lipocortin 1 has recently been cloned and expressed (Wallner et al., 1986) and the purified recombinant molecule used in these experiments was generously provided by the Biogen Research Corporation (Cambridge, MA, U.S.A.). Mononuclear cells or neutrophils were isolated from the peripheral blood of healthy subjects, washed in calcium-free phosphate-buffered saline to remove endogenous calcium and resuspended at a final concentration of 1 × 10⁶ cells/ml in RPMI 1640 medium containing 10% (v/v) fetal bovine serum. Cells were subsequently preincubated for 1 h at 37°C in the presence of increasing amounts of lipocortin 1 (0–30 μg/ml). After a wash step in Dulbecco’s minimum essential medium, Fcγ receptor expression was assessed. The two methods used were: (a) enumeration of erythrocyte–antibody (EA) rosettes formed when leucocytes were incubated at 4°C in the presence of ox erythrocytes sensitized with rabbit anti-ox erythrocyte IgG; (b) binding of monoclonal antibodies (mAb) directed against three distinctively characterized Fcγ receptors designated type I (mAb 32.2), type II (mAb IV.3) or type III (mAb 3G8) (Anderson & Looney, 1986). mAb binding was detected by flow cytometry using an indirect staining technique using the F(ab′)² fragment of fluorescein isothiocyanate-conjugated anti-mouse IgG. Calibration to enable estimation of the number of Fcγ receptors expressed per cell was by the method of Girard et al. (1987).

Abbreviations used: EA, erythrocyte–antibody; mAb, monoclonal antibody.

In a group of four subjects, incubation of mononuclear cells with increasing amounts of lipocortin 1 resulted in a dose-dependent inhibition of EA rosetting with maximum 70% inhibition occurring at 15 μg/ml (Fig. 1a). In a larger group of 12 individuals, incubation of mononuclear cells with this concentration of lipocortin resulted in a decrease in the median number of rosette-positive cells from 18.8 × 10⁴ (range 110.1–30.2 × 10⁴) to 5.5 × 10⁴ (range 0–14.5 × 10⁴), representing a significant fall of 70.8% (P<0.01) (Fig. 1b). A similar pattern of EA rosette inhibition was found for neutrophils, although maximum impairment of 60% occurred at 30 μg of lipocortin/ml. EA rosette inhibition did not occur when cells were incubated with sham preparations purified from Escherichia coli which did not contain the gene for human lipocortin. Also, the effect was abrogated when the preincubation step was carried out in calcium-free medium.

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