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BRIEF COMMUNICATION

Cremophor EL as an adjuvant affecting immunoglobulin class switch in the immune response to the thymus-independent antigen α(1–>3) dextran B 1355 S

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SUMMARY

The humoral immune response to the so-called thymus independent antigen dextran B 1355 S in conventionally raised BALB/c mice consists solely of IgM antibodies. Expression of IgG anti-Dex antibodies in these mice is prevented by pre- or perinatally activated idiotype-specific T-suppressor lymphocytes. IgG B-memory cells nevertheless develop during the course of immunization, but are arrested in an anergic state. In the presence of Cremophor EL the induction of this anergic state is inhibited and the immune response shifts fully to an IgG anti-Dex response.

Cremophor EL (polyethyleneglycolglycerol ricinoleate) is used as solvent for a variety of hydrophobic pharmaceutical and cosmetic compounds, as well as for various poorly soluble drugs administered intravenously. A variety of immunological hypersensitivity reactions to compounds dissolved in Cremophor EL has been described. Among these substances are the anaesthetic drug Althesin,[1] as well as castor oil itself in cosmetic formulations.[2] The adjuvancy of Cremophor EL has also been emphasized by showing that it can enhance, as potently as Freund's complete adjuvant, delayed-type hypersensitivity (DTH) reactions to bovine serum albumin (BSA) or sheep erythrocytes.[3] From these experiments, it seemed that adjuvancy was selective for DTH reactions, since humoral immune responses were not enhanced.

In the following we show that Cremophor EL is a very potent adjuvant for inducing an isotype shift from IgM to IgG antibodies to so-called thymus-independent antigens like α(1–>3) dextran B 1355 S (Dex). In these experiments BALB/c mice, 8–12 weeks of age, were immunized with 10 μg of Dex by intraperitoneal (i.p.) injection of the antigen in aqueous solution. Cremophor EL [325 mg/kg body weight (bw)] was given i.p. daily from Day 2 before immunization for 4 weeks. This Cremophor EL concentration would be approximately the amount given per animal if mice were treated with 50 mg cyclosporin A/kg given in this solvent. In other experiments (data not shown), a 10-fold lower dose of 26 mg Cremophor EL/kg bodyweight led to a comparable adjuvant effect. Dex-specific antibodies were determined at Days 14, 20 and 30 after immunization. They were measured as total (IgM and IgG) antibodies in a haemagglutination assay or as IgG antibodies in an IgG anti-Dex antibody determining indirect ELISA[4] and an isoelectrofocusing (IEF) assay.[5]

The humoral immune response to the so-called thymus-independent antigen Dex in conventionally raised BALB/c mice consists solely of IgM antibodies. In germ-free BALB/c, and in BALB/c nu/nu mice Dex induces a concomitant IgG antibody response.[5] This response is prevented in conventionally raised BALB/c mice by pre- or perinatally activated I-E^d-restricted idiotype-specific T-suppressor lymphocytes which confine the response to IgM antibodies (ref. 4 and data submitted for publication). IgG B-memory cells nevertheless develop during the course of immunization but are arrested in an anergic state from which they can be rescued only under certain conditions, e.g. in adoptive transfer experiments using X-irradiated allotype congenic recipients.[6] In the presence of Cremophor EL the induction of this anergic state is inhibited and the immune response of the immunized BALB/c mice shifts to an IgG anti-Dex response (Table 1) by expressing exactly the public idiotype (pH_I 7·4 spectrotype) characteristic for the BALB/c anti-Dex response (Fig. 1). The target cells affected by the adjuvant effect seem to be T cells. Figure 2 shows that, *in vitro*, neither Dex-specific IgM nor IgG antibody production is affected by 15 μg Cremophor/ml, the dose corresponding approximately to the highest one used *in vivo*. However, proliferation of T cells, represented by the idiotype-specific Ts cell clone 178–4 operating in the anti-Dex response, is strongly reduced. This finding is compatible with previous results showing that Dex-specific B memory cells are arrested in an anergic state under the regulation of idiotype-specific T-suppressor cells.[4–6]

From the above data it is possible that an anti-bacterial immune response which is usually a predominant IgM response

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Table 1. IgG anti-Dex antibody response in BALB/c mice treated with Cremophor EL

	Mean IgG (μg/ml) ± SEM*	
Time	Control	Cremophor EL
Day 14	0·20 ± 0·37	75·31 ± 11·24
Day 30	0·72 ± 0·75	51·74 ± 13·67

* BALB/c mice were immunized at Days 0 and 21 i.p. with 10 μg Dextran B 1355 S. Groups of five animals each were injected with either phosphate-buffered saline (control) or Cremophor EL (325 mg/kg bw) from Day −2 until Day 30. Sera were analysed at Days 14 and 30. Dex-specific IgG antibodies were measured in an indirect ELISA as described in ref.[4] IgM antibody titres varied only twofold in the three groups.

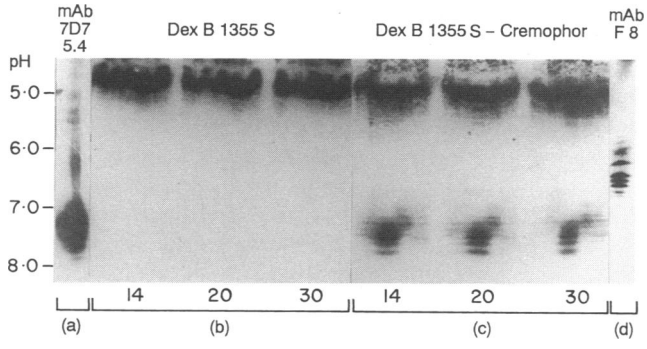

Figure 1. Isoelectrofocusing (IEF) pattern of sera from BALB/c mice immunized with α(1−>3) dextran B 1355 S. Sera correspond to those analysed in Table 1. Animals which were treated with Cremophor EL (c) show a marked IgG anti-Dex response, which is lacking in the control group (b). Two Dex-specific monoclonal antibodies were developed in parallel as marker. 7D7.5.4 carries the public MOPC 104 idiotype (spectrotype pH_I 7·4) (a) and mAb F8 a private idiotype (spectrotype pH_I 6·8) (d). The IEF pattern was visualized by binding of radiolabelled antigen.[5] IgM antibodies do not enter the gel because of size and stain at the top.

can be altered by Cremophor EL such that IgG antibodies become a relevant component of the humoral immune response. In case of antigenic mimicry between bacterial and self-determinants an IgG antibody response, because of potentially hazardous side-effects, could be unwanted.[7,8]

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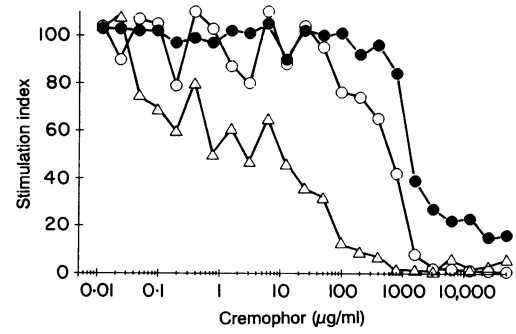

Figure 2. The effect of Cremophor EL on *in vitro* stimulation of B and T lymphocytes. 4×10^6 spleen cells of Dex-primed BALB/c nu/nu mice were co-cultured with various doses of Cremophor EL in Costar wells in 2 ml DMEM with 10% foetal calf serum (FCS). After 2 days of incubation the amount of Dex-specific IgM (●) and IgG (○) was measured in an indirect ELISA.[4] T-cell proliferation (△) was measured by co-culturing 1×10^4 178-4 Ts cells[4] activated with the KT3 (anti-CD3) mAb[9] in the presence of [^{3}H]thymidine.[4] The stimulation index for antibody production and proliferation is the percentage of stimulation with Cremophor/stimulation in absence of Cremophor.

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