CORRELATION BETWEEN ACTIVE ROSETTE FORMATION AND DELAYED CUTANEOUS HYPERSENSITIVITY IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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The effect of encephalitogenic myelin basic protein, BP, on active rosette-forming T cells (ARFC) was compared to that of nonencephalitogenic peptide S42, a synthetic analogue of the tryptophan region of BP. Depression of ARFC by these antigens was reversible within 24 h after a second dose of the antigen into the skin, or after in vitro incubation of lymphocytes with the sensitizing antigen (Ag-ARFC). The ratio of Ag-ARFC to ARFC rose with time following the sensitization but fell shortly before the clinical onset of experimental allergic encephalomyelitis in animals sensitized with BP. In contrast, the Ag-ARFC/ARFC ratios for animals sensitized with peptide S42 reached plateau levels from which they did not drop. The kinetics of the Ag-ARFC/ARFC responses paralleled those for delayed-type skin hypersensitivity (DTH) in the respective animals. The DTH responses rose following sensitization and fell shortly after the appearance of clinical signs of EAE. The results of this study provide in vitro and in vivo evidence for sensitization to myelin basic protein, and focus attention on the ARFC as a measure for an immunologically active cell population which may be quantitated by antigenic stimulation.

INTRODUCTION

In a previous report from this laboratory (1) we have shown a small but statistically significant depression in the active T cell rosetting population.

Abbreviations used in this report: EAE, experimental allergic encephalomyelitis; DTH, delayed-type skin hypersensitivity; ARFC, active rosette-forming T cells; Ag-ARFC, antigen-stimulated active rosette-forming T cells; TRFC, total rosette-forming T cells.
tion (ARFC) in animals sensitized with the encephalitogenic myelin basic protein (BP) or with peptide S42. The amino acid sequence of peptide S42 is analogous to the disease-inducing tryptophan region of the myelin basic protein (2) and is known to induce cutaneous delayed-type hypersensitivity (DTH) which is not followed by clinical or pathological signs of experimental allergic encephalomyelitis (EAE) (3). The depression in the ARFC, which was not related to any changes in the circulating lymphocyte counts, was readily reversible by in vitro incubation of lymphocytes with the sensitizing antigen prior to active rosette formation. The increase in the ARFC was antigen specific, which could not be induced by an unrelated basic protein such as calf thymus histone (1).

Studies have shown that the cutaneous DTH responses to BP were greatly impaired following the appearance of clinical signs of EAE (days 3 and 4). In contrast, the DTH responses to nonencephalitogenic peptide S42 remained at high levels for more than 60 days following sensitization (3). The purpose of this study was to characterize further the antigen-induced ARFC (Ag-ARFC) at timed intervals following sensitization by comparing the Ag-ARFC/ARFC ratios with DTH responses in animals sensitized with encephalitogenic and nonencephalitogenic antigens. Also, ARFC responses were studied in guinea pigs skin tested with the sensitizing antigen 24 h prior to the ARFC assay.

**EXPERIMENTAL PROCEDURE**

*Animals.* Hartley guinea pigs weighing 600–650 g were used. The animals were sensitized with a single injection of 150 µg of BP or 100 µg of peptide S42 as previously described (1).

*Rosette Assays.* Procedures for the preparation of peripheral blood lymphocytes, rabbit erythrocytes, and other reagents used for the rosette assays have been previously described (1). The ARFC and Ag-ARFC subpopulations were estimated by incubating lymphocytes at 37° for 15 min in the absence or presence of 10⁻²¹ to 10⁻¹⁵ mol/liter of antigen, respectively, before the addition of rabbit erythrocytes. The mixture was further incubated for 60 min at 37° as previously described (1).

*EAE and DTH Assays.* Detailed procedures for EAE and DTH assays have been described (5). Briefly, sensitized guinea pigs were shaved with the hair clipper the day before the skin test was done. The antigens were dissolved in sterile physiological saline, and 0.1 ml of the solution containing 15 µg of either BP, peptide S42, or control antigens was injected intracutaneously. Bovine serum albumin, ovalbumin, and calf thymus histone (Worthington Biochemical Corp., Freehold, New Jersey) were used as control antigens. Skin-tested guinea pigs were inspected at 3 and 6 h, and the erythema was recorded at 24 h as the average of the maximum and the minimum diameters for each skin test site. At this time, biopsies of the skin test sites were taken from a representative number of animals for histological examination. To study the effects of the skin-testing antigens on rosette