EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN MICE: Adoptive Transfer of Disease is Modulated by the Presence of Natural Suppressor Cells*

ILENE N. MONTGOMERY AND HELENE C. RAUCH
Wayne State University School of Medicine
Department of Immunology and Microbiology
Detroit, Michigan 48201

Accepted May 25, 1984

Normal, untreated syngeneic recipients of lymphocytes from mice with experimental allergic encephalomyelitis (EAE) do not generally express adoptively transferred disease. Cell transfer of EAE is more successful when syngeneic recipients are treated with cyclophosphamide (CY) prior to the injection of donor cells. Normal, untreated recipients that do not develop EAE after receiving EAE donor lymphocytes are also unresponsive to subsequent encephalitogenic challenge. Those CY-treated recipients that fail to develop EAE after cell transfer do develop EAE after subsequent challenge. After reconstitution with normal splenic lymphocytes, CY-treated recipients do not develop EAE after subsequent challenge. These findings suggest the presence of an intrinsic natural suppressor cell subpopulation in naive mice which modulate the expression of adoptively transferred T lymphocytes.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is a primary demyelinating disease induced in mice by an intradermal inoculation of the encephalitogen (allogeneic or syngeneic CNS tissue) emulsified in complete Freund's adjuvant (CFA) accompanied by intravenous injections of pertussis vaccine. Within two to three weeks following challenge, a majority

* Special Issue dedicated to Dr. Elizabeth Roboz-Einstein.
Address Reprint Request to: Dr. Helene C. Rauch, Wayne State University, Department of Immunology and Microbiology, 540 E. Canfield, Detroit, Michigan 48201.

1399
of the inoculated mice will develop neurologic signs: limp tail, ataxic gait or ascending paralysis. On pathologic examination, inflammatory lesions are observed in the CNS, typically as perivascular cuffs of mononuclear cells principally situated in the white matter. Genetically restricted susceptibility in this T-cell mediated autoimmune disease has been well documented (10).

Adoptive cell transfer of EAE has been demonstrated in syngeneic guinea pigs and rats (14, 11, 15); large numbers of cells are usually required (10^8–10^9). Prior treatment of recipients of either guinea pig and rat cells has not usually been necessary to permit the expression of the adoptively transferred cells. However transfer can be facilitated by culturing donor cells in the presence of myelin basic protein (MBP), the encephalitogenic antigen (4, 5). For example spleen cells do not transfer EAE in rats, unless they are cultured with MBP prior to infusion (5, 12). Recently Bernard (2) succeeded in adoptively transferring EAE in mice; recipients were treated with low dose irradiation prior to EAE donor cell infusion. In our continuing analysis of the regulatory mechanisms in autoimmune neurologic disease, we observed the innate resistance of normal mouse recipients to adoptive transfer of EAE.

To examine the nature of that restriction we have undertaken cell transfer of EAE using both SJL and F₁ (BALB/c × SJL) mice and pretreatment of cell recipients with cyclophosphamide (CY). High doses of CY have a toxic effect on bone marrow precursor cells, but at lower doses, the bone marrow is spared, and apparently only a suppressor T cell population is selectively eliminated (1). Our data suggest the presence of an intrinsic naturally occurring suppressor-like cell population in normal mice which modulates the expression of EAE by adoptively transferred T lymphocytes.

**EXPERIMENTAL PROCEDURE**

*Induction of EAE.* Cell donors, aged 6–8 weeks old, were either SJL males or F₁ hybrid (BALB/c × SJL) males in these experiments. EAE challenge consisted of intradermal footpad injections of 0.2 ml of an emulsion consisting of lyophilized mouse CNS tissue (50 mg/ml saline) in an equal volume of CFA (10 mg H37Rv M. tuberculosis/ml IFA). This was accompanied by an i.v. injection of pertussis (10⁹ organisms) vaccine (Lot C507F, Michigan Department of Public Health) followed by an identical pertussis injection 48 hours later. *Recipients.* Recipients were sex-matched, syngeneic mice, aged 6–8 weeks in all experiments. Recipients were either untreated or treated with cyclophosphamide. The CY was injected i.p. 24 hours prior to transfer at a dose of 100 mg/kg unless otherwise indicated. Those recipients not showing clinical signs of EAE within 18–20 days (unless noted otherwise) of transfer were challenged for EAE as described above.