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Absence of the memory response to encephalitogen following intergender adoptively transferred experimental autoimmune encephalomyelitis

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ABSTRACT

Animals that have recovered from adoptively transferred EAE develop clinical disease signs 2–3 days earlier than controls when challenged with encephalitogen. This may be due to the reactivation of donor-derived memory cells or stimulation of recipient-derived memory cells primed during the adoptive disease episode. In order to determine the origin of the memory cell subset, we used a donor–recipient model where donor cells are rejected in recipients following a course of adoptively transferred disease. Our results suggest the early onset of disease seen in recipients recovered from adoptively transferred disease and challenged with encephalitogen is due to the sustained presence of donor-derived memory cells.

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1. Introduction

The clinical signs of experimental autoimmune encephalomyelitis (EAE) develop in rats and mice following immunization with an encephalitogen emulsified in adjuvant or the transfer of peptide-specific encephalitogenic T cells into syngeneic recipients. Clinical disease is associated with inflammation that is characterized by mononuclear cell infiltration of the central nervous system (CNS) tissue, which is still apparent even after disease recovery. During the disease episode, the inflammatory cell infiltrate and localized immunopathology can result in neuroantigen release and additional encephalitogenic stimuli. Whether this inflammatory milieu, with its associated immunopathology and neuroantigen release, stimulates the development of pathogenic T cells responsive to additional encephalitogenic determinants is difficult to assess directly, although the existence of T cells responsive to additional neuroantigens has been measured by in vitro assays.

While models of actively induced EAE are used to study multiple aspects of immunoregulation, the continuing contribution of the immunostimulatory antigen/adjuvant depot is not easily measured and rarely discussed. Adoptive transfer of lymphocytes from immune donors to naive recipients avoids the issue of a persisting antigen/adjuvant depot within the recipients and in general, such studies have established the cellular nature and the contribution of T-cell subsets to immune responses ranging from protective anti-pathogen immunity to autoimmune disease. The effectiveness of the cell transfer depends on the development or expression of effector function by the transferred cells within the recipient, a response that requires histocompatibility between the cell donor and the recipient. Due to the required donor and recipient histocompatibility, the transferred cells are likely to survive in the recipient for an extended but indeterminate period of time. Consequently, when the nature of the recipient immune response is assessed at a later time point, measured either as effector, regulatory, or memory responses, any interpretation must also take into account the presence and potential contribution of persisting transferred donor cells.

Analyses of the immune status of animals following a clinical disease course of EAE are potentially confounded by influences from the initial disease stimulus, which are either immunization with encephalitogen or adoptively transferred encephalitogenic T cells. In an attempt to overcome these variables, we took advantage of the findings that in some inbred strains of mice and rats, the HY antigen expressed by male cells provides an antigenic stimulus for rejection when transferred into females of the same inbred strain. Such recognition results in the eventual elimination of the transferred male cells (Tyznik and Bevan, 2007). The relatively delayed eradication of transferred male cells following recognition of their HY antigen (7–14 days in some models) allows the transfer and function of encephalitogenic effector cells within the female recipient environment. When the transferred male cells within the female recipient are cleared due to their HY expression, the initial effector population and other cells contained within the infusion are no longer present and no longer contribute to any subsequent measurable

Abbreviations: CFA, complete Freund's adjuvant; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; MBP, myelin-derived basic protein.

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immunologic response parameters within the recipient. This cell rejection phenomenon allows for a clear distinction to be made between a donor-cell-derived vs. recipient-cell-derived contribution to the immunologic parameter under study.

In the rat model of EAE, animals that have recovered from adoptively transferred disease develop clinical signs of disease earlier than control groups following subsequent immunization with encephalitogen (Willenborg and Danta, 1985; Willenborg and Parish, 1988; Bouwer et al., 1990). This early disease development is indicative and attributed to a memory (i.e., previously primed) encephalitogenic cell population, which appears to be a distinct subset from encephalitogen-specific precursor cells (Bouwer and Hinrichs, 1994). It can be envisioned that this encephalitogen-specific memory-cell population may derive from the recipient's endogenous T-cell pool that was stimulated during the adoptively transferred disease episode as a consequence of the associated inflammation and neuroantigen release. Although clinical disease as observed in the rat model of EAE is acute with relapses typically not seen, this concept of inflammation-associated neuroantigen release resulting in the stimulation of additional populations of encephalitogenic T cells is thought to contribute to the development of the relapsing stage of disease in EAE, a phenomenon that is seen primarily in some mouse models following recovery from the primary episode of their clinical disease (Cross et al., 1993; McMahon et al., 2005). Alternatively, the population of encephalitogen-specific memory cells is a component of the initial population of adoptively transferred cells that persist in the recipient, and following neuroantigen challenge, develop into the cells responsible for the earlier onset of clinical disease seen in these cell recipients. The relative involvement of these two possibilities has implications for the development of immunotherapy protocols and reagents to alleviate and or regulate episodes of disease. To distinguish between these two possible explanations in the rat model of EAE, we adoptively transferred encephalitogenic cells from male Lewis rats previously immunized with myelin-derived basic protein (MBP) into either male or female Lewis-rat recipients. Male cells are rejected in the females within 30 days (Dunn, 1975; Chen and Silvers, 1982). When these female recipients of male encephalitogenic cells are challenged with MBP-CFA 30-days post-transfer, a time period where an episode of adoptive disease has occurred and the transferred cells are rejected, the female recipients do not develop accelerated disease, an observation that implies the absence of memory cells. Our observations suggest that the transferred cell population is responsible for the primary disease and is also the source of memory cells responsible for early disease that develops in response to the subsequent challenge with encephalitogen. We find no evidence to support the sensitization of recipient lymphocytes by the inflammation and neuroantigen release intrinsic to a clinical EAE episode that would account for the memory-cell response observed as the early onset of disease following neuroantigen challenge.

2. Materials and methods

2.1. Animals

Inbred female and male Lewis rats (Harlan Sprague Dawley, Indianapolis, IN) were housed and maintained within the animal care facility at the Portland Veterans Affairs Medical Center (PVAMC). The PVAMC Institutional Animal Care and Use Committee approved all animal experiments prior to the initiation of these studies. Animals were provided food and water without restriction and hand-watered during periods of paralysis when necessary.

2.2. Encephalitogen

Myelin-derived basic protein (MBP) was prepared from guinea pig brains according to a previously reported procedure (Diebler et al., 1982).

2.3. MBP sensitization

EAE was induced in 8–10-week-old Lewis rats by the injection of 50 μg MBP emulsified in complete Freund's adjuvant (MBP-CFA) containing 10 mg/ml heat-killed *Mycobacterium tuberculosis* H37Ra (Difco Laboratories, Detroit, MI). A total of 0.05 ml MBP-CFA was injected into each front footpad (Hinrichs et al., 1981).

2.4. Clinical signs of EAE

Animals were evaluated daily for the onset of EAE following MBP-CFA immunization or adoptive transfer by an investigator with no prior knowledge of each group's treatments. Clinical scores of ascending paralysis were assigned based upon the following criteria: flaccid tail, grade 1; hindquarter weakness, grade 2; and complete hindquarter paralysis, grade 3.

2.5. Adoptive transfer

Spleens were obtained on day 12 day following MBP-CFA immunization. Single-cell suspensions were prepared and either directly transferred into naive recipients (typically 1×10^8 cells/recipient, iv), or the splenocytes were stimulated for 72 h with MBP (2 μg/ml, RPMI 1640, 5×10^{-5} M 2-ME, 5% heat inactivated FCS), harvested by centrifugation, washed twice, viable cell number determined, and adoptively transferred at 2×10^7 cells/recipient, iv in a volume of 1 ml (Bouwer et al., 1990).

2.6. Gender sensitization

In some experiments, Lewis rats were primed with 3×10^7 naive splenocytes iv from donors of the opposite gender (or, as a control, of the same gender) prior to their use as recipients of MBP-immune lymphocytes.

2.7. Cell clearance

To determine the fate of male cells following transfer into female recipients, the cervical lymph nodes were collected from naive rats, single-cell suspensions prepared and the washed cell suspensions stained with carboxyfluorescein succinimidyl ester (CFSE) following the manufacturer's instructions (Molecular Probes, Eugene,OR). After staining with CFSE, 3×10^7 cells were injected into each recipient via the tail vein. Two, 4, 7, 10, 14, 20, 30, and 35 days following transfer, the cervical nodes were collected from each of four recipients and processed independently. The number of CFSE stained cells in each of the lymph node suspensions was determined by flow cytometry. Approximately 1% of the recovered lymph node cells were $CFSE^+$ at the first sample time. This percentage was used as the 100% level when determining numbers of $CFSE^+$ cells in recipients analyzed at the later time points. At sample day 30, cells were also isolated from the spleen, liver and CNS for the determination of the presence of CFSE labeled cells.

3. Results

3.1. Adoptively transferred male cells are cleared in female recipients

To assess the fate of adoptively transferred cells, female rats were injected with CFSE-labeled male or female spleen cells. CFSE-labeled male-derived cells are evident in the cervical nodes 14 days following adoptive transfer into female recipients. However, CFSE-labeled male cells are no longer detectable in the cervical nodes of female recipients 30 days following cell transfer (Fig. 1). CFSE-labeled male cells also are not detectable in the spleen, liver and CNS of the female recipients 30 days following cell transfer (data not shown). As a control, CFSE-labeled female derived cells are readily detected in female recipients