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Compartmentalization of TCR repertoire alteration during rejection of an intrabrain xenograft

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Abstract

Xenograft rejections of embryonic pig neural cells implanted into the adult rat striatum occurs within 3–4 weeks, following a dramatic T cell infiltration. Little is known about the cross-talk between the brain and peripheral lymphoid tissues which results in this recruitment and lymphocyte homing. To better characterize the dynamics of the T cell response against xenogeneic neural cells implanted into the brain parenchyma, we used both qualitative and quantitative methods to follow the alterations of the CDR3 length distribution (CDR3-LD) of the TCR (T cell receptor) β chain in the transplanted striatum and compared this response to that observed in the deep cervical lymph nodes, spleen, and blood. Data showed that the T cell repertoire diversity was highly altered in the recipient brain during xenograft rejection. Comparison of the alterations of the CDR3-LD between several animals revealed a single public alteration in the Vβ20 family, and many private alterations of the CDR3-LD which differed from one infiltrated brain to another. Alterations of the T cell repertoire were also observed in lymphocytes homed into the deep cervical lymph nodes. However, they differed from the alterations detected in the infiltrated brains. Conversely, no significant alteration of the CDR3-LD was detected in the spleen or in the blood. These data suggest that the deep cervical lymph nodes play an active role in the process of xenograft recognition or/and rejection. However, they also indicate that the fate of T cells homed in the brain and deep cervical lymph nodes differs.

Keywords: Neuronal transplantation; Rejection; T cell; TCR; Repertoire; Cervical lymph nodes

Introduction

Allotransplantation of fetal human neural cells/tissue is an attractive restorative strategy for patients with focal neurodegenerative disorders, such as Parkinson's disease or Huntington's disease (for a review, see Bjorklund et al., 2003). Implanted neurons can extend axons and achieve a neurochemical differentiation in the host brain and usually are well tolerated in spite of their allogeneic character. This tolerance is due to the immune-privileged nature of the CNS. The unique immune status of the CNS results from the restrictive properties of the blood–brain barrier, the lack of conventional lymphatic drainage, a scarce expression of histocompatibility antigens, and an absence of professional antigen-presenting cells associated with a marked anti-inflammatory potential. However, the brain's immune privilege is not absolute, as it is clearly compromised following implantation of xenogeneic neuronal cells (Brundin et al., 1989; Melchior et al., 2002; Remy et al., 2001). Studies performed with porcine fetal cells derived from the ventral mesencephalon and implanted into the rat striatum indicated that rejection usually occurs within 3–5 weeks

Abbreviations: CDR3-LD, Complementarity determining region 3 length distribution; CNS, Central nervous system; DCLN, Deep cervical lymph nodes; EAE, Experimental allergic encephalomyelitis; MS, Multiple sclerosis; MHC, Major histocompatibility complex; PBL, Peripheral blood lymphocyte; TCR, T cell receptor.

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post-implantation (Melchior et al., 2002). Graft rejection was characterized by an infiltration of lymphocytes together with activated macrophages/microglial cells. Antibodies, complement, NK, and B cells participate in the rejection process, as well (Barker et al., 2000; Larsson et al., 1999, 2001). However, the critical role of T lymphocytes was emphasized by studies showing that treatments inhibiting lymphocytic functions efficiently prolong graft survival. Such immunosuppressive treatments have included the use of cyclosporin A (Brundin et al., 1989; Duan et al., 1995; Pakzaban et al., 1995; Pedersen et al., 1995, 1997; Wennberg et al., 2001), antibodies directed against the TCR, the IL-2 receptor, the CD4 co-receptor, and costimulatory molecules (Honey and Shen, 1999; Larsson et al., 2002, 2003; Okura et al., 1997; Wood et al., 1996). Furthermore, we have previously shown that the cytokine transcripts which rapidly accumulate during rejection mainly belong to infiltrating lymphocytes of the Th1/Tc1 subtypes, and that the proinflammatory context which develops at the level of the graft is consistent with a local amplification of the T cell responses (Melchior et al., 2002).

Antigens are recognized by the TCR, which is generated by gene rearrangements among the constant and variable regions. As a part of the β chain, the association of one of the several V(D)J alleles generates the hypervariable region called complementarity determining region-3 (CDR3). CDR3 hypervariability is further increased by the inclusion of a random number of triplets during these rearrangements. Measurement of the CDR3 length distribution (CDR3-LD) of the different Vβ genes provides an estimation of the antigenic diversity recognized by a T cell population. In addition, quantitative measurements of the β chain TCR transcripts by real-time PCR can allow in vivo identification of dominant peaks having a similar CDR3 length within a specified Vβ family and can indirectly assess the magnitude of T cell selection in a given inflammatory site (Degauque et al., 2004; Guillet et al., 2002).

In the present study, we used qualitative and quantitative approaches to monitor TCR alterations of infiltrating T lymphocytes following implantation of porcine neural xenografts into the striatum of LEW.1A rats and compared the patterns to those found in the draining deep cervical lymph nodes (DCLN), spleen, and blood of the same animals. Our results show that T cells infiltrating the brains of animals engrafted with an identical pool of donor porcine cells display altered CDR3-LD profiles. Moreover, CDR3-LDs obtained from RNAs extracted from the DCLN lacked most of the common features with the profile found in the brain of the corresponding animal. These findings suggest that during the infiltration of the xenograft, T lymphocytes homed into the brain and T lymphocytes found in the DCLN undergo a differential evolution at the same time. Furthermore, remote compartments, such as the peripheral blood or the spleen, are not affected. Such results argue for a loco-regional cross talk involving DCLN in an immune response against an antigen placed into the brain.

Materials and methods

Animals and transplants

Thirty female LEW.1A rats weighing approximately 300 g, obtained from Charles River (Rouen, France), were transplanted bilaterally with 400,000 cells dissociated from the ventral mesencephalon of 28-day-old porcine fetuses. These fetuses were obtained by hysterectomy of two pairs of Large White sows. Fetuses collected from each pair were pooled and served to transplant 15 rats. Therefore, rats formed two groups of 15 animals (referred to as series A and B), which differed solely by the origin of the donor cells. Sows were inseminated and maintained in a station of the National Institute of Agricultural Research (Nouzilly, France) and were sacrificed in an accredited slaughterhouse. Embryos were transported and dissected in Hank's balanced salt solution (Gibco BRL, Cergy-Pontoise, France). Pieces of ventral mesencephalon were kept in hibernation medium for 2–3 days before dissociation as described previously (Remy et al., 2001). LEW.1A rats were anesthetized and implanted bilaterally, according to an experimental procedure and stereotaxic coordinates previously described (Melchior et al., 2002; Remy et al., 2001) and treated in compliance with the ethical rules of our Institute. Because the moment at which graft infiltration takes place cannot be determined in the absence of behavioral test or neuroimaging, five animals randomly chosen were sacrificed at 21, 28, and 35 days after transplantation. No sham-transplanted animals were done in this study as we have previously shown that no T cell infiltration was detected in the month following surgery (Melchior et al., 2002).

Transplant analysis

For both series of animals, a portion of brain, mainly composed of the striatum, which encompassed the graft implanted in the left hemisphere, was dissected and dropped in liquid nitrogen for RNA extraction. In the second series of transplanted animals (series B), blood was harvested by intracardiac puncture in heparinized tubes before sacrifice and lymphocytes were separated using a Ficoll gradient. Spleen and DCLN, located deeply between jugular arterial/vein and the trachea, were also collected and placed in liquid nitrogen before storage at −80°C. To evaluate the infiltration of the neuronal xenograft by immunohistochemical analysis, the right hemisphere of all animals (series A and B) was first gently frozen in isopenthane at −35°C and stored at −80°C. Then sections of 14 μm were prepared with a Leica cryostat and kept at −80°C. For immunohistochemistry, sections were fixed with chilled 4% paraformaldehyde for 15 min and processed as described in previous studies (Remy et al., 2001). Infiltration was assessed according to pre-established criteria. These criteria included the presence of infiltrating activated macrophages/microglial cells (as revealed with the OX42 monoclonal antibody from Serotec, Kindlington,