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Adoptive Transfer of T Lymphocytes Sensitized against the Prion Protein Attenuates Prion Invasion in Scrapie-Infected Mice

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There is to date no effective way of preventing or curing neurodegenerative diseases such as Alzheimer disease or transmissible spongiform encephalopathies (TSEs), or prion diseases, constitute a well defined set of fatal neurodegenerative disorders including Creutzfeldt-Jakob disease, scrapie, bovine spongiform encephalopathy, and chronic wasting disease (1–3). TSEs are primarily characterized by their transmissibility irrespective of their etiology and by the presence in the brain, and in most instances also in secondary lymphoid tissues, of the scrapie prion protein (PrPSc), which is misfolded and is partially resistant to proteinase K (PK) (4, 5). PrPSc is generated by the conversion of a ubiquitous, host-encoded, membrane-bound glycoprotein, cellular prion protein (PrPc) (6). PrPc is remarkably conserved through evolution and is present in all mammal species (7). However, its function in health is still debated (8–10). PrPSc is presumably the causative agent of prion diseases and is responsible for neurological lesions and disease transmission by the template conversion of host PrPc (11–13). In the peripherally acquired forms of TSE, PrPSc accumulates first in the germinal centers (GCs) (14, 15), notably in follicular dendritic cells (FDCs) (16) and dendritic cells (17–19) before invading the CNS.

TSE agents do not spontaneously elicit Ab- or cell-mediated responses (20). Still, on the basis of studies of amyloidosis and Alzheimer disease (21–23) and of reports showing that Abs prevent PrP conversion in vitro (24–26), several teams have attempted to generate active or passive immunity against PrP in scrapie-infected mice. Passive Ab transfer (27, 28), transgenic constitutive secretion of anti-PrP IgM Abs (29), or active immunization with whole PrP (30, 31), PrP peptides (32, 33), PrP fragments (34), or DNA constructs encoding Prnp sequences (35–37) have resulted in reduced invasion of secondary lymphoid organs, delayed onset, prolonged survival, and, in a few instances, definitive remission. None of these approaches is, however, totally satisfactory. Ab injections must be given in large amounts continuously and become ineffective once the CNS is invaded (28) and the tolerogenicity of PrPc limits the efficacy of active vaccination. Adoptive cell transfer could be an alternative (38), as it would supply the patient with substantial amounts of long-lived effector cells. For human therapy, these cells could be selected and expanded in vitro culture. From an experimental point of view, adoptive transfer of selected lymphocyte subsets may contribute to...
a better understanding of the defense mechanisms at work against TSE and to a realistic evaluation of the autoimmune risk.

Because mice made PrP deficient by gene ablation (Prnp<sup>−/−</sup>) (39) are not tolerant to the protein and allow the free development of anti-PrP T and B cell precursors, they have been largely used to generate immune responses against the prion protein (40–45). In parallel, DNA vaccination has been successfully developed by several teams to elicit Abs against PrP (46–49). By combining both approaches, we have previously reported that Prnp<sup>−/−</sup> mice, when challenged weekly with recombinant DNA, responded reproducibly and vigorously against PrP (50).

Responses included sensitized CD4<sup>+</sup> helper T cells predominately reacting against an I-A<sup>B</sup>-restricted epitope mapped at position 156–170 of mouse prion protein, as well as Abs recognizing plastic-bound recombinant and membrane-bound native PrP. PrP<sup>−/−</sup>-deficient mice are totally unresponsive to recombinant DNA, thereby confirming that the expression of PrPc on host tissues induces a deep tolerance in both B and T cells (20, 44, 51). On the basis of these results, we have developed models in which the lymphocytes collected in DNA-immunized Prnp<sup>−/−</sup> mice were transferred into histocompatible C57BL/6 recipients that were totally or partially devoid of their own lymphocytes for better colonization. In the present study, we analyzed the capacity of PrP primed lymphocytes to delay the peripheral propagation of prions in mice infected by an i.p. route. Results show that such cells retard scrape progression in secondary lymphoid organs and the brain, provided that they are periodically reactivated with Ag. Interestingly, protection does not appear to be mediated by Ab.

**Adoptive cell transfers**

T cells were enriched by negative magnetic selection with Dynal kits (Invitrogen) according to the manufacturer's recommendations. T cell purity, as assessed by flow cytometry, was between 90 and 95%.

Ten million T cells or total spleen cells were injected i.v. into 6- to 8-week-old recipients. Engraftment was monitored on days 15 and 30 in PBMC and on days 30, 60, and 90 in lymph nodes (LN) and spleens. Subset analysis was made with anti-CD3, anti-CD19, anti-CD4, and anti-CD8 Abs (BD Pharmingen). Events were acquired in a FACSCalibur flow cytometer and analyzed with CellQuest Pro software (all from BD Biosciences).

**Proliferation assay**

T cells, adjusted at 3 × 10<sup>4</sup>/well in flat-bottom microtiter plates (Falcon; BD Diagnostic Systems) were cocultured in triplicate with 3 × 10<sup>5</sup> mito-mycin C-treated spleen cells as APCs (Sigma-Aldrich) and peptide at 6, 2, and 0.6 μM. Plates were incubated for 5 days at 37°C and 5% CO<sub>2</sub>, receiving 1 μCi of [3H]thymidine (Amersham/GE Healthcare) for the last 18 h. Incorporated radioactivity was measured by scintillation in a MicroBeta 1450 Trilux (Wallac). The index of proliferation is the ratio of the average cpm of triplicate cultures containing T cells, APCs, and peptide vs the average cpm of cultures with T cells and APCs but without peptide.

**ELISPOT**

T lymphocytes were plated onto nitrocellulose-based microwells (Millipore) together with mitomycin C-treated spleen cells as APCs and peptide at different concentrations. Plates were incubated at 37°C with 5% CO<sub>2</sub> for a period of 24 h for IFN-γ and 48 h for IL-4. Spots of IFN-γ were detected with anti-R4-6A2 as the capture Ab and biotinylated XMG1.2 for revelation (BD Pharmingen). The capture and revelation Abs for IL-4 were from the IL-4 ELISPOT kit (R&D Systems). Alkaline phosphatase conjugated to streptavidin was reacted with tetrachloridophenyldophosphate/tetrazolium nitroblue as substrate (Roche). Spots were counted with an automated ELISPOT plate counter (AID Diagnostika).

**Detection of Abs against native PrPc**

Ab binding to native PrPc was measured by indirect immunofluorescence, as described previously (45, 50, 55), on a stably transfected EL4 T cell clone activated overnight with anti-CD3 Ab (clone 2C11 at 10 μg/ml) to maximize cell surface PrPc. Cells, blocked first for Fc receptors, were incubated for 20 min with serum dilutions at 1/10, 1/50, and 1/100, washed in FACS buffer, and revealed for 20 min with clone Mark1, a FITC-conjugated rat anti-mouse κ-chain Ab. Alternatively, to increase sensitivity the bound Ab was revealed in two steps, first with a biotin-linked anti-mouse IgG<sub>Fc</sub> κ Ab and then with streptavidin APC (both from BD Pharmingen). Results are expressed as geometric mean fluorescence intensity (MFI) measured by flow cytometry. Every assay included samples of normal mouse serum and the monoclonal anti-PrP Ab SAF83 as negative and positive controls, respectively.

**Detection of PK-resistant PrP by Western blotting**

Mice were infected i.p. in a 0.1-ml volume with dilutions of a pretreated stock homogenate of 139A prion in PBS, prepared from a pool of brains of sick mice culled at terminal stage. Inoculated doses are given in LD<sub>50</sub> units.

Spleens collected 60 days postinfection (dpi) in one protocol and 90 dpi in another were homogenized with the Ribolysen method (Hybaid) and adjusted at 10% (w/v) in PBS plus a mixture of antiproteases. Aliquots were mixed with 4% sarkosyl in PBS (pH 7.4) for 10 min at 37°C and then treated with 2.5 U of benzonuclease (Sigma-Aldrich) at 37°C for 30 min and subsequently precipitated by addition of 4% trichloroacetic acid with 0.5% sodium phosphotungstic acid (Sigma-Aldrich) at 37°C for 30 min. Samples were centrifuged at 2 × 10<sup>8</sup> × g for 30 min at 37°C. Samples were run using 10% SDS-PAGE in the first and 12.5% SDS-PAGE in the second. PrP was revealed with Ab SAF83 at 1/3000 dilution or Ab SAF84 at 1/5000. The signal was captured on autoradiographic film in the first protocol and by a Fujifilm LAS3000 camera in the second. Spot quantification, in the second protocol, was made on the diglycosylated band, the most prominent one, using a Fujifilm software program. Blots were exposed for 3 min. Signal intensity was expressed in arbitraries units. Western blots on brain were performed according to the same protocol, with a precipitation step in sodium phosphotungstic acid. Ten milligrams of brain homogenate were loaded. In some experiments, brain samples were spiked with...
air dried, fixed in acetone at 4°C, and stored at -80°C in a liquid nitrogen system. Ten-micrometer sections were deposited on SuperFrost Plus slides, included inguinal, brachial, and mesenteric nodes and contained from 15 to 25 lymphocytes. The volume was delivered through a mesodermal 30-gauge needle with an automatic Hamilton dispenser. Each homogenate was injected into five or six recipients. We chose the average time of onset rather than the terminal point to be checked, the percentages and absolute numbers of T lymphocytes between day 15 and day 30 was seen in blood samples collected on days 15 and 30 from the same individual mice. B, Percentages of B cells (white bars), CD4+ T cells (black bars), and CD8+ T cells (gray bars) in LNs of three CD3ε−/− mice ± SD 30, 60, and 90 days after transfer and a C57BL/6 control ± SD for each time point. LN pools included inguinal, brachial, and mesenteric nodes and contained from 15 to 25 × 10^6 cells per mouse. C, Numbers of B cell and T cell CD4+ and T cell CD8+ lymphocytes in spleens from CD3ε−/− mice 30, 60, and 90 days after transfer. Spleen size evolved from 75 to 90 × 10^6 cells at day 30 to 90 to 10^6 cells at day 90. D, Lymphoid microarchitecture and presence of FDC in a CD3ε−/− mouse 90 days after transfer of 1 × 10^7 primed T cells. The GC is captured at an original magnification of ×100 and the enlarged photograph of the circled area is at an original magnification of ×400. The green arrow points at an FDC characterized by the presence of multiple nuclei with pale chromatin, contrasting with the dark chromatin of adjacent lymphocytes.

**Histology**

GC microarchitecture was analyzed on fixed 5-μm serial sections of spleens stained with H&E and examined at various magnifications. Microphotographs were taken with an Olympus BX51 microscope equipped with an Olympus DP50 camera.

Lymphoid reconstitution of RAG-2−/− mice was assessed on 10-μm-thick frozen sections stained with biotinylated CD19 Ab plus Texas Red-streptavidin for B cells, FITC-conjugated CD3 Ab for T cells (BD Pharmingen) and Texas Red-biotinylated FDC M2 clone for FDCs (ImmuNoKontact).

Infiltrating T cells were detected on brain hemispheres cut crosswise into three equal segments and snap frozen in a Tissue-Tek embedding system. Ten-micrometer sections were deposited on SuperFrost Plus slides, air dried, fixed in acetone at 4°C, and stored at -20°C until use. CD4+ T cells were revealed with an appropriate dilution of an anti-CD4 Ab coupled to FITC (BD Pharmingen). Slides were mounted in Vectashield fluorescence-mounting medium containing 4′,6-diamidino-2-phenylindole for confirmation of cell integrity (Vector Laboratories). Photomicrographs were taken with a Leica SP2 confocal microscope and analyzed with Leica Confocal software. Magnifications are indicated with scale bars. T cell counting was performed at ×200 original magnification on an Olympus BX 61 fluorescence microscope associated with a digital camera.

**Statistics**

Statistical analyses were made with GraphPad software. Results were considered significant for p < 0.05.

**Results**

*T lymphocytes sensitized against the prion protein expand in wt recipients and overcome peripheral tolerance*

Before evaluating the protective potential of sensitized lymphocytes, we analyzed their capacity to stably engraft and remain Ag responsive in PrP-expressing recipients. Primed T cells generated in Prnp−/− mice by three weekly injections of naked DNA were collected 1 wk after the last challenge and injected at 1 × 10^7 cells into PrP+−/−, CD3ε knockout mice. Lymphocyte reconstitution was monitored on days 15 and 30 in PBMC and on days 30, 60, and 90 in LN and spleens. A significant expansion of T cells between day 15 and day 30 was seen in blood samples from individual mice (Fig. 1A). Simultaneous lymphoid restoration in LN and spleens 30, 60, and 90 days after transfer was observed (Fig. 1, B and C). By day 90, the last time point to be checked, the percentages and absolute numbers of T cells in LN and spleens were, however, still below the values of normal C57BL/6 mice tested in parallel, confirming that in mice with no thymic output, such as CD3ε−/− mice, exogenous T cells remain confined to the activated memory compartment and cannot repopulate the compartment allotted to naive T cells freshly exported from the thymus (56).
Fig. 2 shows the architecture of a CD3ε−/− GC 90 days after transfer. At a higher magnification one can see a typical multinucleated FDC. Similar pictures were seen 30 and 60 days after T cell transfer (data not shown). Thus, transferred mice do not display microarchitecture anomalies likely to retard prion progression.

T cell sensitization was monitored at the time of transfer on spleen aliquots from individual Prnp−/− donors assayed for their capacity to specifically proliferate in coculture with loaded APCs. All samples reacted to PrP158–187, a 30-mer peptide encompassing the major CD4+ epitope, but not to PrP68–97, which contains no known epitope (Fig. 2A) (50).

With the idea that periodic Ag recalls may enhance the reactivity of engrafted T cells, a subgroup of recipients was periodically challenged with PrP158–187. A 30-mer peptide encompassing the major CD4+ epitope, but not to PrP68–97, which contains no known epitope (Fig. 2A) (50).

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FIGURE 3. PK-resistant PrPSc and infectivity titers in spleens of mice adoptively transferred with T lymphocytes. A, Mice nos.1 and 2 received primed T cells subsequently boosted with PrP158–187, mice nos.3 and 4 received primed/nonboosted T cells, and mice nos.5 and 6 received unrecalled naive T cells. The symbols for plus (+) and minus (−) refer to PK-treated and non-treated samples, respectively. B, Spleen infectivity measured in the same groups of mice by in vivo titration on tga20 mice. Numbers designate the same spleens in A.

the same level of pathological protein as the basic controls (Fig. 3A). PrPSc reduction could not be directly quantified on Western blots but could be estimated by titrating infectivity in the same spleen samples. As shown in Fig. 3B, spleens that displayed minimal accumulation of PrPSc by Western blotting contained ~10 times less ID50 infectivity than spleens from recipients of unrecalled T cells or from the control group (p = 0.043 by Kruskal-Wallis variance analysis and p < 0.05 by Dunn’s two by two test between groups 1 and 2).

T cells injected at the time of infection still attenuate lymphoinvasion

The next experiment was performed with the double purpose of finding out whether protection was still provided when infection and transfer took place simultaneously and whether protection could be demonstrated in normal, mildly irradiated (450 rad) and transfer took place simultaneously and whether protection could be demonstrated in normal, mildly irradiated (450 rad) C57BL/6 mice. Recently irradiated recipients were infected with 2 × 105 LD50 units of 139A at day 1 and received 1 × 107 primed T cells on the following day. Two subgroups were constituted, one challenged with PrP158–187 and the other left unchallenged. Controls were irradiated or nonirradiated C57BL/6 mice infected at the same time. As shown in Fig. 4A, control spleens assayed at 90 dpi displayed similar amounts of PrPSc by Western blotting whether they had been irradiated or not. Conversely, spleens of mice transferred with PrP-primed T cells and boosted displayed considerably less pathological PrPSc, notably when compared with spleens of primed/nonboosted T cell recipients (Fig. 4B). Quantification of Western blotting signals showed significant difference between the spleens of boosted and nonboosted mice (p < 0.05, by unpaired two tailed Student’s t test) (Fig. 4C). Brains of the same subgroups were probed by Western blotting to find out whether neuroinvasion had also been attenuated by adoptive transfer. No PrPSc could be detected in the CNS by this method at 90 dpi, even after loading high concentrations of protein (equivalent of 10 mg of brain) and exposing the blots longer than usual (20 min instead of 3) (data not shown). PrPSc started to be detected in brain homogenates spiked with 1% of terminally sick brain and was fully evident at 10%, indicating the limits of PrPSc detection by Western blotting (data not shown).

Adoptive transfer of primed and boosted splenocytes attenuates clinical disease

The effect of adoptive cell transfer on clinical disease was addressed in the next experiment. RAG2−/− mice, reconstituted with total primed splenocytes, were infected with 2 × 105 LD50 units of 139A 30 days postreconstitution. Five mice were periodically boosted with PrP158–187 starting at the time of cell transfer as described in the above experiments, and five received PBS in adjuvant. Controls were RAG2−/− mice restored with naive splenocytes. Blood samples collected 30 days after transfer showed a sizable percentage of T lymphocytes and, to a much lower extent, B lymphocytes (Fig. 5A). Pepsin boosting had no apparent impact on lymphocyte expansion.

As Fig. 5B shows, scrapie onset in recipients of primed and boosted splenocytes was considerably delayed (236 median days) compared with median onset in recipients of primed/non boosted cells (193 median days) and controls (194 median days). The difference of 42 days was statistically significant at p < 0.01 by multivariate log rank test. Terminal stage (Fig. 5C) was similarly protracted in recipients of primed and boosted spleen cells (284 median days vs 233 and 235 days in the two other groups). The difference was significant by multivariate log rank test (p < 0.05).

Because the integrity of GC architecture is crucial for peripheral progression of prions (57, 58), we verified that the reconstitution of B and T cell zones and the FDC network had properly occurred in the group in which disease had been attenuated. As shown in Fig. 5D–F, the follicle of a mouse that had received primed T cells with boosts was fully reconstituted 6 mo after transfer. This rules out the possibility that scrapie retardation was the consequence of abnormal GC morphology as proposed in other situations in which scrapie was delayed (59).
Absence of significant Ab response in recipients of primed lymphocytes

Because it is generally assumed that Abs, notably those recognizing native PrPc, block scrapie progression (27, 29, 60), we considered the possibility that adoptive cell transfer worked through Ab mediation. Ab against PrPc was measured using a previously described immunofluorescence assay on transfected EL4 cells overexpressing PrPc (50, 55). Fig. 6A shows fluorescence values generated by

FIGURE 4. PrPSc detected by Western blotting in spleens of wt C57BL/6 mice i.p. infected with \(2 \times 10^5\) LD_{50} UNITS of 139A and simultaneously transferred with primed T cells. A, Western blots of spleen controls from C57BL/6 mice irradiated or not irradiated and infected at the same time as experimental mice. B, Western blots of spleens from three mice transferred with primed and boosted T cells (the four left lanes) or from three mice that received the same batch of primed/nonboosted T cells (the three right lanes). The symbols for plus (+) and minus (−) refer to PK-treated and non-treated samples, respectively. C, Signal quantification of the major diglycosylated bands shown in B. Light gray diamonds represent transferred/boosted mice, and black diamonds represent transferred/nonboosted mice. Differences are statistically significant by Student’s t test.

FIGURE 5. RAG-2^{−/−} mice transferred with PrP-primed and -boosted total spleen cells develop an attenuated disease. A, Reconstitution 30 days after transfer of T cells (in white) and B cells (in gray) in PBMCs of individual RAG-2^{−/−} mice injected with primed splenocytes boosted twice with PrP158–187 or not boosted. B, Scrapie onset in recipients of naive unrecalled spleen (\(n = 6\); white dots, hatched connecting line), primed/nonboosted spleen (\(n = 5\); gray dots, hatched connecting line), or primed and boosted whole spleen (\(n = 5\); black dots, full line). Mice were infected i.p. 30 days after transfer with \(2 \times 10^4\) LD_{50} units of 139A. C, Terminal stage in same groups of mice (same numbers, same legend as in B. D–E, Cryosections of a spleen follicle from a RAG2^{−/−} mouse 6 mo after cell transfer and boosts showing B cell zones (D), T cell zones (E), and the FDC network (F).
FIGURE 6. Ab against native PrPC in adoptively transferred mice. A. Sera collected 30 days after cell transfer into CD3ε−/− mice. Dark dots are individual recipients of primed/boosted T cells, medium gray dots are recipients of primed/nonboosted T cells, and white dots are recipients of naive unchallenged T cells. Gray diamonds are sera collected in DNA-immunized Prnp−/− donors of T cells at the time of transfer. Sera were assayed at 1/10 and 1/50 dilutions and are shown at 1/10. The horizontal lines show the geometric means. B. Same as in A, with sera from infected C57BL/6 wt mice collected at 90 dpi. MFI values are globally higher due to the revelation in two steps with biotin-linked Ab and streptavidin. They are shown at a dilution of 1/50. Boxes show the range of MFI values and the whiskers represent the 95% interval of confidence. The black box represents the sera of immunized Prnp−/− donors of T cells (12 samples), primed/boosted T cells (11 samples), primed/nonboosted T cells (10 samples), and normal mouse sera (eight samples). C. Sera collected at days 30 and 90 after transfer in recipients of primed and boosted (five samples) or primed/nonboosted splenocytes (five samples) and DNA-immunized Prnp−/− donors (eight samples).

sera collected from CD3ε−/− mice 30 days after transfer of naive, primed/boosted, or primed/nonboosted T cells. In Fig. 6A, MFI values of sera from the original DNA-immunized Prnp−/− donors are also shown. Whereas MFI values of donor sera were all in the third log, those of transferred mice were at the limit of detection (ranging from 10 to 20), with no significant differences between groups. MFI values shown in Fig. 6B are from sera collected in normal, mildly irradiated C57BL/6 mice 90 days after the transfer of primed/boosted or primed/nonboosted T cells. To increase the sensitivity of the assay, bound Ab were revealed in two steps, including a biotinylated secondary Ab and fluorescent streptavidin. MFI values of sera from transferred mice were slightly higher than those in Fig. 6A, ranging from 20 to 50, but were not significantly different from values of normal sera and were still considerably lower than those measured in immunized primed−/− donors. The pattern of MFI values was identical in RAG2−/− recipients of primed total spleen cells despite the fact that the transfer included primed T and B cells (Fig. 6C). The average fluorescence was not significantly different whether recipients of primed splenocytes had been boosted or not. The modest and not statistically significant MFI decline between sera collected in the same mice on days 30 and 90 suggests that a limited number of Ab-secreting B cells had been brought within the inoculum but did not thrive in the hosts. Considering the possibility that Ab is produced in transferred and boosted mice but is rapidly removed from the circulation by binding with PrPC, we examined the kidneys of such mice for the presence of high Ig deposits. Glomeruli sections of those mice and of mice challenged with PBS in adjuvant only showed slightly brighter fluorescence than unmanipulated C57BL/6 controls, due probably to Freund's induced nephrotoxicity (61). However, there was absolutely no difference in fluorescence intensity between the two former groups (data not shown). It therefore seems unlikely that Ab scarcity in sera is due to its rapid precipitation onto PrPC.

**Primed/boosted T cells infiltrate the host brain more readily**

To find out whether disease attenuation was correlated with an accumulation of infiltrating lymphocytes in the CNS, brains isolated at the terminal stage from culled RAG2−/− mice previously transferred with primed/boosted or primed/nonboosted spleen cells were histologically examined for the presence of CD4+ T cells. The brain of a C57BL/6 mouse developing myelin oligodendrocyte glycoprotein-induced experimental autoimmunity encephalomyelitis (EAE) served as positive control (62). As illustrated in three photomicrographs (Fig. 7A), the brain of a recipient of primed/boosted spleen cells contained substantially more infiltrating T cells than the brain of a recipient of primed/nonboosted cells. CD4+ T cell frequency was most elevated in the brain of a mouse developing EAE. Infiltrating T cells were counted in anterior, median, and posterior brain sections of two recipients of primed/boosted cells, two recipients of primed/nonboosted cells, and two age-matched C57BL/6 healthy controls (Fig. 7B). CD4+ T cells were significantly more abundant in every brain section of mice that had received primed/boosted spleen cells and had developed a delayed form of scrapie, suggesting an in situ involvement of the adoptively transferred T cells. Variance analysis of number of cells/field in cumulated areas gave values of $p < 0.001$ between the three groups and $p < 0.01$ for recipients of primed/boosted spleen cells vs primed/non boosted spleen cells by Bonferroni’s two by two test.

**Discussion**

Our interest in adoptive cell transfer is double. First, adoptive cell transfer provides a way of analyzing the contribution of defined lymphoid cell subsets in the immunotherapy of neurodegenerative conditions and a way of realistically measuring the autoimmune risk against self-Ag concentrated in the CNS. Second, it represents an alternative strategy to passive injection of Ab or active immunization with immunogenic formulations of PrP, both to date limited in their effects. At variance with Abs that must be continuously renewed, sensitized lymphocytes can be stably engrafted and iteratively reactivated for long-term treatments. Adoptive cell transfer of sensitized lymphocytes from Prnp−/− donors into wt recipients is obviously not a clinical option. Its main justification is to understand the mechanisms of immune protection at work in TSE and to provide proofs of concept. In a translational perspective of human adoptive therapy, the patient’s own T lymphocytes would be collected, expanded by in vitro culture, and reinfused...
A first conclusion from the present study is that T cells alone are sufficient to retard prion propagation in secondary lymphoid organs of infected mice. In two independent experiments, PrP-primed purified T lymphocytes have been shown to slow down the accumulation of pathohomal PrPSc in the spleen and to reduce infectivity, irrespective of whether lymphocytes were administered before infection or simultaneously or whether recipients were T cell deprived or T cell replete (Figs. 3 and 4 respectively). Whether neural invasion is similarly retarded by adoptive T cell transfer could not be inferred from these experiments, because progression in the brain at 90 dpi is probably just beginning and Western blotting is not sensitive enough to detect amounts of pathological protein that are too low. Spiking experiments actually show the limits of this assay. The issue of neuroinvasion was addressed in a specific experiment in which clinical disease was followed until terminal stage. Mice transferred with primed and boosted total splenocytes developed an attenuated form of scrapie. Onset was delayed by 42 days and the terminal stage was extended to 236 days as compared with 194 days in controls or in nonboosted recipients of sensitized spleen cells. Such a difference in disease duration corresponds to 2 log infectivity by i.c. titration (67). Morphological anomalies of GC architecture and the FDC network were clearly not responsible for the observed delay. Recipients of primed and boosted splenocytes showed fully reconstituted spleen follicles with distinct T and B cell zones and FDCs. Interestingly, the amount of PrPSc detected by Western blotting in the brains at the time of sacrifice was the same irrespective of disease duration (data not shown). The degree of neurodegeneration leading to terminal stage appears to be the same for all mice; it is only the time needed to reach that threshold that differs between protected and unprotected mice.

A second conclusion is that T cells need to be periodically reactivated to delay lymphoinvasion. There is an apparent link between protection and the capacity of engrafted T cells to secrete lymphokines upon encounter with a PrP peptide. Loss of function in the absence of antigenic challenge might be a manifestation of peripheral tolerance. Because memory T cells are more resistant to peripheral tolerance than naive T cells (68), they are not physically eliminated and may respond more efficiently than naive T cells following 90 days of engraftment with periodic recalls. Tolerance mediated by regulatory T cells (Tregs) is another important pathway of peripheral tolerance (69). Tregs are probably not imported within the cohort of T cells that comes from PrP-deficient donors, but they may develop in PrP-sufficient hosts and may affect nonboosted T cells more heavily than regularly challenged T cells. A recent study from our laboratory demonstrated the development of such regulatory T cells, notably when mice were infected with 139A (55).

Finally, the third main conclusion is that attenuated lymphoinvasion is not accompanied by a significant Ab response against PrPc. One may thus wonder whether the Ab is a major protagonist of protection in adoptive cell transfers as observed in other forms of immunotherapy against TSE. A contribution of cell-mediated immunity conveyed by sensitized T cells may be at least as important. Abs against native PrPc were globally scarce in all categories of adoptively transferred mice, as compared with the Abs detected in the sera of DNA-vaccinated Prnp−/− donors. The modest and statistically nonsignificant MFI decline between days 30 and 90 in the sera of RAG2−/− mice receiving PrP-primed splenocytes (Fig. 6C) suggests that a few Ab-secreting B cells may have been transferred but did not persist in the long term. Several studies show that anti-PrP B lymphocytes are strictly controlled in PrP-sufficient wt mice (40, 44, 51). This would explain why imported B cells have a limited survival in RAG2−/− recipients and why transferred T cells have difficulties in establishing productive cooperation with host B cells that have differentiated in a PrP-positive environment and have therefore been made tolerant.

In view of the low levels of Ab detected in adoptively transferred mice and of the absence of significant MFI differences between protected and unprotected mice, one is tempted to favor the second alternative, namely an implication of cell-mediated immunity. This is further supported by the fact that primed/boosted T cells with a protective potential more readily cross the blood brain barrier with FITC Ab.
barrier and infiltrate the host CNS. One may infer from this observation that T cell boosting enhances the expression of a broad spectrum of chemokines, chemokine receptors, and adhesion molecules that facilitates T cell passage through the blood brain barrier (70). It cannot be definitively concluded that the presence of infiltrating T cells in the brain is directly related to the protective advantage, but it is tempting to speculate that lymphocytes with such properties will reach more easily the sites where prions expand, first in the lymphoid organs and secondarily in the CNS.

Two independent studies have shown that macrophages reduced prion infectivity during early infection (71, 72), probably by degrading infectious prions. In a more recent study, it was found that mice deprived of TLR-4 developed accelerated scrapie, irrespective of which prion strain was injected and whether prions had been inoculated i.c. or i.p. (73). Prions therefore seem able to initiate innate immune responses that can counteract their progression. It is conceivable that in the course of adoptive cell transfer, PrP-primed T cells encounter Ag, release lymphokines, and activate macrophages and other phagocytes. A similar scenario might take place in the CNS, with primed T cells crossing the blood brain barrier and activating local microglial cells or patrolling macrophages, thus harnessing innate immunity to adaptive immunity.

In conclusion, the present results show that adoptive cell transfer of PrP-sensitized lymphocytes, notably T cells, is a realistic option for preventing or treating TSE. This method can be improved by increasing the effectiveness of factor interest equipped with the appropriate set of chemokines and cytokines. On one hand, the possibility for primed/boosted CD4+ T cells to infiltrate the brain more abundantly than under natural conditions (74, 75) calls for caution to avoid the pitfalls experienced in Alzheimer disease (76, 77). On the other hand, their propensity to cross the blood brain barrier can also be interpreted as a positive argument in favor of adoptive cell transfer, provided the tenuous frontier that separates beneficial immunity from deleterious autoimmunity can be controlled.

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Disclosures

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vaccination delays or prevents prion infection via an oral route. *Neuroscience* 133: 413–421.


