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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. The idea of treating those conditions by immunological approaches has progressively emerged over the last ten years. Encouraging results have been reported in Alzheimer disease and in peripheral forms of mouse prion diseases following passive injection of Abs or active immunization against the peptides or proteins presumably at the origin of those disorders. Still, major difficulties persist due to some characteristics of those conditions such as slow evolution, brain location, uncertainties regarding precise pathogenic pathways, and, above all, the fact that the target Ag is self, meaning that it is poorly immunogenic and potentially harmful if tolerated was transgressed. To analyze some of those difficulties, we are developing adoptive cell transfer approaches. In this study, lymphocytes sensitized against the prion protein in nontolerant Prnp−/− mice were transferred into compatible wild-type recipients which were partly or totally devoid of their own lymphocytes. Under such conditions, we found that the engrafted T lymphocytes resisted peripheral tolerance, remained reactive for several months against epitopes of the prion protein, and significantly attenuated the progression of prions in secondary lymphoid organs with subsequent delay in the evolution of the neurological disease. Interestingly, those protective T lymphocytes secreted lymphokines and migrated more readily into the host CNS but did not appear to be engaged in cooperation with host B cells for Ab production. The Journal of Immunology, 2009, 183: 6619–6628.

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 PrP (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. Abs 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 by 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−/−) (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−/− mice, when challenged weekly with recombinant DNA, responded reproducibly and vigorously against PrP (50). Responses included sensitized CD4+ helper T cells predominantly reacting against an I-Aβ-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-sufficient 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−/− 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-induced lymphocytes to delay the peripheral propagation of prions in mice infected by an i.p. route. Results show that such cells retard scrapie 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.

Materials and Methods

Mice

Prnp−/− mice were derived from the original Zurich stock on a 129Sv× C57BL/6 background (39). The null mutation was backcrossed in our facility for 12 generations on C57BL/6 mice. Compatibility between mutated and wild-type (wt) mice was verified by reciprocal skin grafting (data not shown). CD3e−/− (52) and RAG-2−/− (53) mutants were on a C57BL/6 background. Other strains used in this study were transgenic zo20 mice (54) expressing multiple copies of PrP and wt C57BL/6 mice. Animals were bred and maintained under strictly monitored specific pathogen-free conditions. Experiments were conducted in compliance with French legislation and European Union recommendations.

Antigens

Naked DNA was produced in competent TOP10 Escherichia coli bacteria (Invitrogen) by amplification of a pcDNA3.1 plasmid (Invitrogen) in which a full-length mouse Prnp sequence was inserted. Endotoxin-free, high quality DNA was prepared with an EndoFree Gigaprep kit (all from Invitrogen). PrP58−187, and PrP68−97 are two 30-mer peptides belonging to a library of 13 overlapping peptides originally designed for identifying CD4+ T cell epitopes in an I-Aβ context. They were synthesized by NeoMPS with a minimum of 80% purity. The two peptides used in this study were soluble at 0.1 mM in water and were LPS free.

Immunizations

Prnp−/− mice were immunized as previously described (50) by three injections at 1-wk intervals of 100 µg of recombinant pcDNA1 plasmid in which the full mouse Prnp sequence has been inserted. The dose was split for injection into both tibialis anterior muscles, which had been presensitized 5 days earlier by cardiotoxin from cobra venom 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 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 tetrachloroindolylphosphate/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 F(ab′)2 and then with streptavidin APC (both from BD Pharmingen).

ELISPOT

T lymphocytes were plated onto nitrocellulose-based microwells (Millipore) together with mitomycin 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% CO2, 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.

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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 LD50 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 the addition of 4% sodium phosphotungstic acid (Sigma-Aldrich) at 37°C for 30 min. Samples were centrifuged at 2 × 105 × g, and pellets were resuspended in PBS plus 0.1% sarkosyl. Aliquots equivalent to 6 mg were PK-digested (proteinase K; Roche) or left undigested. In one protocol, digestion was performed with 10 µg/ml PK at 37°C for 5 min; in the other, PK was used at 50 µg/ml for 30 min at 37°C. Samples were run using 10% 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 arbitrary units. Western blots on brains 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

Adaptive 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-wk-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 × 105/well in flat-bottom microtiter plates (Falcon; BD Diagnostic Systems) were cocultured in triplicate with 3 × 106 mitomycin 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% CO2, 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.
FIGURE 1. Lymphocyte reconstitution in PBMCs, LNs, and spleens of CD3ε−/− mice transferred with 1 × 10⁷ T cells. A, T cell expansion measured 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 × 10⁶ 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 × 10⁶ cells at day 30 to 90 × 10⁶ cells at day 90. D, Lymphoid microarchitecture and presence of FDC in a CD3ε−/− mouse 90 days after transfer of 1 × 10⁷ 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.

0.1%, 1%, and 10% of a brain homogenate of terminally sick mice. Blots were exposed for 3 and 20 min.

Titrations of infectivity

Spleen infectivity titration in tgg20 mice as described by Fischer et al. (54). Twenty-microliter aliquots of spleen homogenates at 10% (w/v) in PBS were injected intracerebrally (i.c.) in Flouthane-anesthetized mice. 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 stage because neurological symptoms, mainly ataxia, appear suddenly and quite homogeneously in tgg20 mice. ID₉₀ units were computed from the relation y = −6.74x + 83.73, where x is the amount of ID₉₀ units in log present in 20 µl of inoculum and y is the time of incubation in days. Ordinate and slope had been previously established using a pretitrated homogenate of 139A.

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 in 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 acetonate 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-diamidinol-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 × 10⁷ 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).
FIGURE 2. Adoptively transferred T cells resist peripheral tolerance. A, Proliferative responses of T cells from DNA-vaccinated Prnp−/− mice at the time of adoptive transfer. The dark bars are proliferation indexes in triplicate ± SD at three concentrations of PrP158-187, and the light gray bars represent responses to PrP68-97, an irrelevant 30-mer peptide. B, Proliferation indexes of T cells after 90 days of engraftment in CD3ε−/− recipients. Dark diamonds show proliferative indexes of T cells that have been periodically boosted with PrP158-187, and the light diamonds are from unrecalled T cells. Horizontal lines mark the mean in each group, and differences between the two groups are significant according to Student’s t test. C, Frequency of IFN-γ spots, 90 days after transfer, in primed T cells boosted with PrP158-187 (left portion of histogram) or primed/non boosted T cells (right portion). Dark gray bars measure the spots generated in the presence of the relevant peptide PrP158-187, medium gray bars measure spots in the presence of irrelevant PrP68-97, and the white bars are spots generated in the absence of peptide. D, Same as in C, with spots of IL-4. E, Proliferative responses, 90 days after transfer, of primed (left portion of histogram) or naive T cells (right portion). Black bars show the response to relevant PrP158-187, and light gray bars show responses to irrelevant PrP68-97.

Fig. 1D 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 in CFA/IFA according to the schedule described above, divided into two subgroups. One was periodically challenged with PrP158-187 in CFA/IFA according to the schedule described above, and the other received PBS in CFA/IFA. Basic controls were CD3ε−/− mice receiving naive T cells that were left unrecalled. All three groups were infected i.p. 30 days after transfer with 4 × 10^4 LD50 units of 139A scrapie. To assess protection provided by T cells, we took as experimental endpoints the amount of pathological PrP in spleens at 60 dpi and infectivity. Spleens of mice transferred with primed T cells displayed minimal amounts of PK-resistant material. By contrast, spleens of mice transferred with primed/nonboosted T cells contained almost called mice. IL-4 producers in unrecalled mice were more abundant but were not specific for PrP158-187, and their frequency was not proportional to peptide concentration.

In a last pilot experiment, we compared naive vs activated T cells transferred in parallel and subsequently challenged with PrP158-187. Naïve T cells were considerably less responsive after 90 days of engraftment than activated T cells (Fig. 2E), suggesting that they were probably less resistant to peripheral tolerance.

Adoptive transfer of primed T cells attenuates prion accumulation in secondary lymphoid organs and reduces infectivity

In a first experiment aimed at assessing protection against scrapie, CD3ε−/− mice were transferred with purified, primed T cells and divided into two subgroups. One was periodically challenged with PrP158-187 in CFA/IFA according to the schedule described above, and the other received PBS in CFA/IFA. Basic controls were CD3ε−/− mice receiving naive T cells that were left unrecalled. All three groups were infected i.p. 30 days after transfer with 4 × 10^4 LD50 units of 139A scrapie. To assess protection provided by T cells, we took as experimental endpoints the amount of pathological PrP in spleens at 60 dpi and infectivity. Spleens of mice transferred with primed T cells boosted with PrP158-187 displayed minimal amounts of PK-resistant material. By contrast, spleens of mice transferred with primed/nonboosted T cells contained almost
C57BL/6 mice. Recently irradiated recipients were infected with 
PrP158–187, mice nos. 3 and 4 received primed/nonboostered T cells, and mice nos. 5 and 6 received unirradiated naive T cells. The symbols for plus (+) and minus (−) refer to PK-treated and non-
treated samples, respectively. B, Spleen infectivity me-
sured in the same groups of mice by in vivo titration on 
tga20 mice. Numbers designate the same spleens in A.

**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, while mice nos. 3 and 4 
received primed/nonboostered T cells, and mice nos. 5 and 6 received unirradiated naive T cells. The symbols for 
plus (+) and minus (−) refer to PK-treated and non-
treated samples, respectively. B, Spleen infectivity me-
sured 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 min-
imal accumulation of PrPSc by Western blotting contained ~10 
times less ID50 infectivity than spleens from recipients of unre-
called 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) 
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. Con-
trols 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 trans-
ferred with PrP-primed T cells and boosted displayed considerably 
less pathological PrPSc, notably when compared with spleens of 
primed/nonboostered 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 
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 concen-
trations 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 ter-
minally 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 col-
lected 30 days after transfer showed a sizable percentage of T lymph-
ocytes and, to a much lower extent, B lymphocytes (Fig. 5A). Pep-
tide 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 possi-
B
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
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 Prnp−/− donors. The pattern of MFI values was identical in RAG2−/− mice 30 days after transfer of primed/nonboosted cells, and two recipients of primed/boosted cells, two recipients of primed/nonboosted cells, and two age-matched C57BL/6 healthy controls (Fig. 7B). 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...
(63). Reactive lymphocytes would be possibly oriented toward the most effective and less harmful Th profile and TCRs engineered for new specificities and higher affinity by genetic transfer of re-arranged TCR α/β chains (64–66).

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 pathological 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 re-activated 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 non-boosted 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 boundary with FITC Ab. Microns length. Arrows point at T cell membranes labeled with FITC Ab. Numbers of infiltrating CD4+ T cells counted in anterior, median, and posterior brain sections. Numbers of infiltrating T cells in EAE are 5–10 times higher.
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 frequency of effectors of 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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