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Cell-based immunotherapy of prion diseases by adoptive transfer of antigen-loaded dendritic cells or antigen-primed CD4+ T lymphocytes

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Abbreviations: PrPc, cellular prion protein; PrPSc, scrapie prion protein; TSE, transmissible spongiform encephalopathies; CNS, central nervous system; AD, Alzheimer disease; TCR, T-cell receptor; scFv, single chain antibodies; DCs, dendritic cells; APC, professional antigen presenting cell; MHC, major histocompatibility complex; Th cell, T helper cell; NK cell, natural killer cell; IFN, interferon; IL, interleukin; T reg, regulatory T cell; CAR, chimeric antigen receptors

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Prion diseases are neurodegenerative conditions caused by the transconformation of a normal host glycoprotein, the cellular prion protein (PrPc) into a neurotoxic, self-aggregating conformer (PrPSc). TSEs are ineluctably fatal and no treatment is yet available. In principle, prion diseases could be attacked from different angles including: blocking conversion of PrPc into PrPSc, accelerating the clearance of amyloid deposits in peripheral tissues and brain, stopping prion progression in secondary lymphoid organs, reducing brain inflammation and promoting neuronal healing. There are many indications that adaptive and innate immunity might mediate those effects but, so far, the achievements of immunointervention have not matched all expectations. Difficulties arise from the impossibility to diagnose TSE before substantial brain damage, poor accessibility of the CNS to immunological agents, deep immune tolerance to self-PrP and short term effects of many immune interventions contrasting with the slow progression of TSEs. Here, we discuss two approaches, inspired from cancer immunotherapy, which might overcome some of those obstacles. One is vaccination with antigen-pulsed or antigen-transduced dendritic cells to bypass self-tolerance. The other one is the adoptive transfer of PrP-sensitized CD4+ T cells which can promote humoral, cell-mediated or regulatory responses, coordinate adaptive and innate immunity and have long lasting effects.

The Present Situation

The demonstration, ten years ago, that antibodies could block the propagation of prion infection in vitro, in infected cell lines[1,2] and in vivo in scrapie-bearing mice[3-5] generated great enthusiasm. Parallel results obtained against Alzheimer disease (AD)[6] or Parkinson disease[7] further supported the idea that vaccinal strategies which had been historically so successful against classical infectious diseases could be similarly applied to neurodegenerative conditions. Ten years later, in spite of numerous encouraging reports, there is some questioning regarding the possibility to propose efficient and safe immunotherapy against neurodegenerative proteinopathies, notably against transmissible spongiform encephalopathies (TSEs). The obstacles have been well identified and extensively reviewed.[8,9] Self-tolerance to PrPc is the main concern. Due to the strong tolerogenicity of PrP, the T-cell and B-cell repertoires which escape tolerance and are available for vaccination appear to be considerably impaired, both quantitatively and qualitatively. Immune selection operates evidently against the lymphocytes that are the most self-reactive, which means those which recognize PrP epitopes with high specificity and affinity and which would be the most effective therapeutically. This explains why it is difficult to raise robust vaccinal responses against PrP and to achieve solid and durable

protection. In most trials of active immunization, the attack rate is unchanged and life extension remains modest. As already mentioned, passive transfer of antibodies generated in PrP-deficient mice was the starting point of immunotherapy in TSEs. Efforts have been pursued since then, but have encountered limited success due to the difficulty to make antibodies cross the meningial barrier and penetrate into the brain at a stage when prions have already invaded the nervous tissue. Therefore, the treatment is effective only if started concomitantly with peripheral infection or very shortly after. Antibodies used in those assays were directed against PrPc and selected for their epitope specificity[10] or their pharmacological qualities.[11] In order to overcome the problem of brain accessibility, antibodies were also administered through intra-ventricular infusion with an osmotic pump, resulting in onset delay and disease attenuation with some prion strains even when treatment was initiated a few weeks after inoculation.[12] Antibodies specific for the 37/67 kDa laminin receptor which acts as a cell surface receptor for prions were also used with definite success.[13] To overcome the problem of blood brain barrier crossing, some groups have engineered single chain Fv antibodies (scFv) which penetrate more easily into the brain owing to their lower molecular weight.[14,15] The latest development in this field has been the insertion of scFv antibody genes into specific viral vectors such as adeno-associated viruses in order to achieve antibody delivery within the CNS.[16-18] Here again, scrapie is slowed down, even after direct prion inoculation into the brain, which constitutes a definite progress, but ultimately all mice succumb to the neurological lesions.

In order to translate immunotherapy into clinic, strategies must be therefore substantially improved. Improvement may come from different directions. A first one should be to strengthen the response of the available anti-PrP repertoire. Ingenious procedures of immunization have been developed including the use of potent adjuvants[19] and of highly immunogenic bacterial and viral vectors encoding the *Prnp* gene sequence,[20-22] but there is probably still room for progress. Second, one should consider other protective pathways, beside antibodies, notably when the disease is fully established in the brain. For instance it might be more pertinent to skew the immune response toward a cell-mediated profile which would associate activated $CD4^+$ T cells with recruited macrophages and microglial cells rather than aiming at getting a humoral response which hardly reaches the CNS. Third, it may be necessary to supply the failing immune system with lymphocytes enriched for more avid anti-PrP T-cell receptors (TCR) and secreting chemokines and cytokines of interest. This could be done by selecting and expanding lymphocytes in vitro before reinfusing them into the host or even by engineering the expression of new antigen receptors. Here, we describe two cell-based strategies which have been developed with success in cancer immunotherapy and which respond to the criteria suggested above. One is the use of antigen-pulsed dendritic cells (DCs) for vaccination, a strategy which should potentiate the immune response generated by the available immune repertoire, with the possibility to reorient responses to a desired mode. The other is the adoptive transfer of PrP-primed T helper cells which could serve as a study model to understand what type of response would be most effective at a given stage of disease evolution. It could also be a clinical alternative to adoptive antibody therapy by providing the host immune system with relatively long-lived, good quality memory effector cells. The two strategies will be described and discussed in the light of recent results obtained in our laboratory.

Vaccination with Antigen Loaded DCs

The process of antigen presentation to T lymphocytes is absolutely central to the genesis of immune responses. DCs, macrophages and B lymphocytes are the main professional antigen presenting cells (APCs). Among them, DCs have the unique capacity to activate naive T cells, thereby initiating primary immune responses.[23,24] Optimal T-lymphocyte activation depends on a series of signals. Signal 1 is antigen-specific; it is delivered through the interaction between the antigen-MHC complex on the APC and the TCR. Signal 2 is a costimulatory signal delivered by APCs and aimed at amplifying signal 1 which is insufficient by itself to activate T lymphocytes and, if not complemented by signal 2, leads to an aborted response. Because DCs express high quantities of MHC class I and class II products together with a wide range of costimulatory molecules such as CD86, CD80 and CD40, they are the most efficient APCs to provide signals 1 and 2 to T lymphocytes.[25] In addition, DCs secrete a panel of cytokines (signal 3) which skew the differentiation of $CD4^+$ T cells toward distinct lineages.[26] Naive T helper cells (Th0) may differentiate into T helper type 1 (Th1) T cells which produce proinflammatory cytokines, mediate cellular immune responses, cooperate with $CD8^+$ T cells for cytotoxic responses and mobilize effectors of innate immunity. Alternatively, Th0 cells may differentiate into Th2 T cells which cooperate with B cells for antibody production. They may also differentiate into Th17 T cells which induce strong inflammatory responses or into regulatory T cells (Treg) whose function is to prevent deleterious autoimmunity. The choice of lineage depends essentially on the presenting DC, which integrates, via a wide range of receptors, an ensemble of physical, chemical and biological messages including the presence and the origin of a pathogen or the distress of surrounding tissues.[27-29] DC precursors migrate from bone marrow to peripheral tissues where they capture, process and present at their cell surface antigenic peptides associated to MHC class I or II molecules. DCs mature and subsequently migrate to the T cell zones of secondary lymphoid organs where they come into contact with antigen-specific T lymphocytes and initiate their activation and lineage commitment.[30]

DCs play a major role in vaccination. In classical vaccination designs, DCs are randomly targeted in vivo by the antigen usually injected with an adjuvant.[31] However, in more recent protocols, DCs can be specifically targeted in vitro. DCs are differentiated and expanded from bone-marrow, peripheral blood or cord blood precursors.[32-34] They are subsequently matured and loaded with the antigen of interest and reinfused into the

original donor. Specific targeting avoids the random delivery of antigen to irrelevant DC or to APC populations which might anergize rather than activate T cells or skew them toward an undesirable type of response, for instance pro-inflammatory Th17 T cells instead of Th2 T cells. By targeting DCs in vitro, the antigen under the right formulation is delivered only to the relevant APCs appropriately matured.[28] Vaccination with targeted DCs has been so far mainly developed against cancer. In a majority of instances, the objective was to raise $CD8^+$ cytotoxic T cells killing tumor cells expressing tumor specific antigens in a MHC class-I context.[35] Several clinical studies have shown encouraging results against diverse types of cancers including melanoma, renal carcinoma[36,37] and colon or lung cancer.[38] More recently, some laboratories have succeeded in using DC vaccination in order to generate MHC class-II restricted $CD4^+$ T-cell responses against tumors. Those studies underlined the necessity for the $CD4^+$ T cells to cooperate with $CD8^+$ T cells for an efficient eradication of tumor cells, both in mice and humans.[39-41] But more recently, it was shown that $CD4^+$ T cells can eliminate melanoma cells on their own, in the absence of $CD8^+$ T or of natural killer (NK) cells.[42]

Those encouraging results have prompted us to transpose DC vaccination to prion diseases. Two MHC class-II restricted 30-mer peptides of PrP (PrP_{98-127} and $PrP_{158-187}$) had been previously identified as potent stimulators of $CD4^+$ T cells when directly injected with adjuvant into PrP-deficient (*Prnp*$^{-/-}$) mice, but not into wt mice naturally tolerant to PrP.[43] We first asked whether DCs pulsed with these peptides would overcome tolerance in wt mice. The answer was positive. Mice twice injected with peptide-pulsed DCs develop $CD4^+$ T cells which proliferate and produce cytokines (IL-4 and IFNγ) in an antigen-dependant way.[44] Of note, consistent with previous observations made in *Prnp*$^{-/-}$ mice, DCs loaded with $PrP_{158-187}$ elicit a Th1 and Th2 response with production of IFNγ and of IL-4 whereas the response elicited by DCs loaded with PrP_{98-127} is exclusively centered on IL-4, suggesting that the choice of the peptide may determine the type of immune response. Antibodies recognizing epitopes expressed on native PrPc are detected in parallel. A detailed analysis of the interactions between the three partners—DCs, T cells and B cells—shows that DCs make direct and independent contacts with the T and the B cell partners. T cells recognize peptide/MHC class-II complexes resulting from the processing of the loaded peptides whereas B cells recognize conformational epitopes of PrPc expressed on the DC cell surface. There is no overlap between the T and B epitopes and both peptide and membrane PrPc are necessary to achieve full cooperation.

Next, we inoculated i.p., a scrapie agent (strain 139A), into mice which had been twice challenged with PrP_{98-127}-pulsed DC and were subsequently boosted every month. Two out of ten mice never became sick and showed no PrPSc in their brain. The eight other mice developed scrapie, but with a longer incubation and longer survival than controls.[44] The total disease duration was extended by 20% whereas it was only around 10% in most reported studies. Interestingly, the duration of the prolongation was correlated with the intensity of the antibody response measured 45 days after infection. The two mice which were definitively protected displayed the highest antibody titers.

The advantage of using DC as a vaccine support was also demonstrated in a recent study performed in the laboratory by Rosset and colleagues.[45] There, instead of being pulsed with antigen, DCs were transduced with a recombinant adenovirus. Mice which had received several boosts of antigenic DCs were subsequently infected with 139A. The best protection was achieved with DCs transduced with a recombinant adenovirus encoding the sequence of human PrP. The conferred protection was considerably higher than when similar recombinant adenoviruses were directly injected in vivo. Both peptide-pulsed and virally-transduced DCs have their advantages and drawbacks. Loading DCs with pure and well-defined synthetic peptides is simple and safe, but it requires the identification of the PrP peptide for a given MHC class II context. Viral transduction of DCs with a full length PrP gene requires an additional step of virus production and might be therefore less safe or less easy to perform, but the peptide sampling and insertion onto MHC class-II products is done by the DC.

Thus, the use of in vitro matured and targeted DCs as a vaccine vector presents definite advantages over direct in vivo delivery of antigen. DCs evoke good T- and B-cell responses including antibodies against native epitopes of PrPc which have the capacity to prevent PrP conversion and hence to block prion expansion. Incidentally, this result shows that B-cell precursors of potential therapeutic interest are not totally absent from the repertoire which has gone through tolerance selection. Even though the B cells which escaped tolerance are probably rare and of mediocre avidity, they may have a chance to expand by antigen stimulation and to improve antibody affinity by somatic mutations of their immunoglobulin genes.

Adoptive T-Cell Transfer

$CD4^+$ T lymphocytes play a key role in adaptive immune responses. They respectively orchestrate cell-mediated, cytotoxic, humoral, inflammatory or suppressive responses through their ability to differentiate into a wide range of functionally different and even opposite lineages, such as Th1, Th2, Th17 or Treg.[46-48] As already mentioned, the decision of lineage commitment comes in the first place from the DC which tailors the response that will be best adapted to the situation.[30] Quite importantly, the decision can be changed either by modifying the conditions under which DCs mature or by reprogramming $CD4^+$ T helper cells.[49]

$CD4^+$ T cells have been so far neglected in the context of imunotherapy against TSE. One reason is that the main focus has been put on B cells and antibodies. The other is that $CD4^+$ T cells have been seen more as foes than as friends following the complications of cell-mediated meningoencephalitis generated by the vaccination of AD patient with Aβ peptide.[50] But the shortcomings of passive antibody transfer which is effective only against the transmitted forms of TSEs and at the early phase of lymphoinvasion, underline the necessity to develop alternative strategies. Adoptive T-cell therapy

could be one of them. CD4+ T cells might exert anti-prion effects in different ways. As Th2 T cells, they may help B cells to differentiate into antibody-producing plasmocytes, induce immunoglobulin switch and contribute to the affinity maturation of antibodies. Thus, supplying the host immune system with a cohort of efficient Th2 helper cells might improve the quantity and the quality of secreted therapeutic antibodies. CD4+ T cells with a Th1 or a Treg profile could be also helpful at more advanced stages of disease, and notably against sporadic and familial forms of TSE which start developing in the brain. Via the secretion of pro-inflammatory cytokines and of chemokines, proinflammatory T cells might attract macrophages into the brain and might activate microglial cells which could clear amyloid deposits, degrade clusters of infectious PrPSc and facilitate neuronal repair. Conversely, Treg lymphocytes might soothe brain inflammation by releasing TGFβ or IL-10. The crucial issue here is to identify precisely which type of CD4+ T cells is most effective at a given stage of disease evolution while avoiding putative autoimmune complications.

To begin investigating those questions, we have developed a very basic study model in which the CD4+ T cells come from PrP-deficient mice, not tolerant to PrP and therefore able to generate full-fledged anti-PrP responses and recipients are histocompatible mice expressing PrP and susceptible to scrapie.[51] We have first asked how long CD4+ T cells sensitized against PrP can survive and remain functional after engraftment into recipients expressing PrP. The results were highly encouraging as T cells appeared to resist peripheral tolerance; they could still proliferate and secrete cytokines, three months after transfer, provided that they were regularly recalled with antigen. We then asked whether such lymphocytes would protect infected mice. This was done under various conditions, in mice made partially or totally lymphopenic, with cell transfer performed before or just at the time of infection. In all situations, T cells primed and iteratively boosted attenuated disease progression. The attack rate was not reduced as with DC vaccination, but there was a substantial life prolongation. An important point to be underscored is that circulating antibodies against native PrPc were low, actually close to background, in all experimental settings. This means that resident B cells did not readily respond to the help brought by the transferred T cells and that protection may not be necessarily mediated by antibodies.[51]

Many important issues remain to be investigated. So far the transferred T cells have been directly collected from immunized *Prnp*$^{-/-}$ mice without polarization toward a specific profile. Those T cells are probably a mixture of Th1 and Th2. It will be essential to examine the consequences of transferring pure lineages of Th1 or Th2 T cells as well as Treg and Th17 T cells. Another major issue is to find out the therapeutic window of adoptive T-cell transfer. The time limit for antibody therapy is apparently around 30 days after infection with a mouse adapted prion strain.[4] It will be important to see whether T cells can still be beneficial when injected beyond this point, notably at the clinical onset or in mice which have been infected by intracranial route. One should also find out whether the risk of adverse autoimmune reactions does really exist or is a simple inference from what has been reported in AD.

To increase the power of our study model, we have recently generated a TCR transgenic mouse which expresses a high frequency of PrP-specific CD4+ T cells. A first analysis of the mouse phenotype shows that anti-PrP TCRs come out of the thymus and populate the periphery only in *Prnp*$^{-/-}$ mice (manuscript in preparation). Central negative selection contributes therefore significantly to the shaping of the CD4+ PrP-specific T-cell repertoire. The T cells carrying the transgenic TCR are highly responsive to PrP and can be expanded in cell culture without prior in vivo priming. They represent an ideal source of lymphocytes for generating activated effector T cells with a polarized profile and for examining the consequences of their adoptive transfer into healthy or infected recipients. Adoptive transfer of T cells from immunized *Prnp*$^{-/-}$ donors into PrP-positive recipients is clearly not transposable to clinical situations, but it is a useful model for answering important methodological questions.

The protocols for TSE patients should be modeled on those currently developed in cancer immunotherapy.[52,53] Assuming that a substantial number of PrP-specific T helper lymphocytes with TCRs of reasonable affinity survive central and peripheral tolerance, they could be submitted to in vitro antigen-driven selection, polarization and expansion before being reinjected into the patient. If it appears that the number of good affinity T helper cells is not sufficient, one should consider the possibility of transducing T cells either with physiological MHC-restricted TCR genes conferring high avidity[54] or alternatively, with non MHC-restricted chimeric antigen receptors (CAR) which express at the cell membrane an antigen receptor constituted by the variable domains of an anti-PrPc antibody.[55] The use of CARs would present the advantage of being totally independent of the MHC class-II context of the patient, and therefore not to necessitate the identification of fitting peptides, as would be the case for physiological α/β TCRs.

Perspectives

Neither DC vaccination nor adoptive T-cell therapy can be translated into clinic at the present stage. The attack rate is modestly reduced with DC vaccination and still not with adoptive T-cell transfer. Life extension is in the order of 20% only, which is obviously insufficient. However one can easily foresee how both strategies might be substantially improved. The most urgent point is to identify the type of adaptive immune response: humoral, cell-mediated, inflammatory, inflammatory plus innate or regulatory, which will be best adapted to the state of disease advancement, to the clinical status of the patient, and most probably too, to the prion strain. On the one hand, our results show that the level of antibodies generated by DC vaccination correlates with protection. On the other hand, protection in mice receiving adoptively transferred T cells is not accompanied

by the production of antibodies. Thus the question of humoral versus cell-mediated immunity is totally open. To identify which mode of response is most efficient might be achieved in a basic study model such as the one that we are currently developing between *Prnp*$^{-/-}$ and wt mice. In a second stage, mice humanized for PrP and for the lymphoid system[56] should be used to probe human prions. Once the type of beneficial immune response is clearly defined, either DCs or T cells can be matured, differentiated or reprogrammed so as to skew the immune responses toward the most helpful profile while avoiding harmful autoimmunity.

Beside engineering good affinity TCRs or CARs on the patient T cells, it might be advisable to transduce DCs or CD4$^+$ T helper cells with genes encoding for cytokines, chemokines and chemokine receptors. Such mediators would facilitate the migration of cells to the sites of interest: gut lymphoid system, draining lymph nodes and brain. For DCs, they would potentiate their impact upon T and B cells; for CD4$^+$ T cells, they would enhance their capacity to cross the brain barrier and recruit agents of innate immunity. The experience accumulating in cancer immunotherapy will be most useful for developing rational and effective immunointerventions against TSE. One may as well anticipate that the lessons drawn from prion diseases will benefit other neurodegenerative conditions such as Alzheimer and Parkinson diseases.

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