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Dendritic Cells And Oral Transmission Of Prion Diseases

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Abstract

Transmissible spongiform encephalopathies (scrapie, BSE, Kuru) develop as CNS diseases after long incubation periods, and many of which may arise following the consumption of infected material. The infectious agent is thought to be a mis-folded form (PrPSc) of a normal host protein (PrPC), which is relatively resistant to proteolytic degradation and which serves as a template, directing host PrP to accumulate in the misfolded form. Animal experiments have shown that CNS disease is preceded by a period in which the agent accumulates in secondary lymphoid organs (Peyer’s patches, lymph nodes, spleen), particularly follicular dendritic cells in the B cell areas of these organs. How the agent is transmitted from the intestinal lumen to the FDCs is largely unknown. Dendritic cells (DCs, cells quite distinct from FDCs) are cells that are specialised to acquire antigens from peripheral tissues and to transport them to secondary lymphoid organs for presentation to T and B lymphocytes. We have shown that DCs can acquire PrPSc from the intestinal lumen and deliver it to mesenteric lymph nodes. In this review we discuss the different stages involved in the migration of PrPSc from the intestine to FDCs and consider the different stages and barriers involved in this process. We conclude that transport of the causative agent, using PrPSc as a biomarker, from the intestine to FDCs is a very inefficient process, which may help to account for the apparent low frequency of individuals who have consumed infected material that go on to develop clinical disease.
**Key words:** Dendritic cells, Prion, TSE, Scrapie, Oral transmission, pathogenesis.

**Key to abbreviations:** CNS: central nervous system; DC: dendritic cell; FDC: follicular dendritic cell; GALT: gut-associated lymphoid tissues; LP: lamina propria; nvCJD: new variant Creutzfeldt-Jacob disease; PK: Protease K; PP: Peyer’s patch; PrP: prion protein; PrP\(^{C}\): cellular isoform of PrP; PrP\(^{Sc}\): scrapie associated PrP; TSE: transmissible spongiform encephalopathy; TSEA: TSE agent.

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1. Introduction: Prions and the oral transmission of TSE

The oral route of infection has been implicated in the pathogenesis of transmissible spongiform encephalopathy (TSE), a disease that targets clinically the central nervous system (CNS). Spread of the disease, both naturally and experimentally, by ingestion of the infectious agent has been described in a variety of species ranging from domestic, as well as wild-life, animals to man [1-10]. The likelihood of spread of the bovine form of TSE (BSE) [11] to human (nvCJD) through the food-chain [6] has aroused long-lasting public health anxiety with adverse economical and political consequences, both in the UK and abroad for the last decade [12-14]. Due to the long incubation period of the illness, infected individuals may remain asymptomatic for years. It remains still highly uncertain as to how risky it has been, or how safe it is now, to eat beef or even meat from other animals likely to carry and pass on the mysterious ‘mad cow’ disease [7, 15].
The disease has also led to the proposition of a novel mechanism of infectivity: that is an infectious agent (TSEA) possibly devoid of nucleic acid. This was first indicated by its refractoriness to UV irradiation [16]. It is also complicated by the many other unusual physical and biological properties of the molecules including, most worryingly, its extraordinary resistance to conventional sterilization procedures. The exact nature of the etiologic agent has been, and still is, a focus of scientific debate.

Although the possible causal agent being an “unconventional slow virus” (Virino Theory) [17] is not excluded [18], live virus of this kind has not been isolated. The lack of specific immune response to the puzzling agent [19], and the absence of inflammatory cell infiltrate in the affected brains, seem to suggest otherwise [20, 21].

PrP<sup>Sc</sup>, also known as ‘scrapie-associated prion protein’, is an abnormally folded, self-encoded protein (PrP<sup>C</sup>) that co-purifies with TSE infectivity [22, 23]. The molecule is now considered to be the crucial, if not the only agent (Protein Theory), responsible for the infection [24]. Structurally, PrP<sup>Sc</sup> is rich in β-sheets, in contrast to the α-helix-rich PrP<sup>C</sup>, and is insoluble in many detergents. The current hypothesis suggests that the pathogenesis involves a key step of prion protein conversion and replication in which PrP<sup>Sc</sup> converts the normal protein, PrP<sup>C</sup>, into the abnormal form [25]. The agent replicates in this way by a post-translational alteration of the tertiary structure (conformational change), but not amino acid sequence, of the host-encoded protein, turning it into a highly self-aggregating, protease-resistant pathological isoform [26, 27]. The “Protein only” theory [28, 29] is supported by evidence from transgenic and knockout studies that mice devoid of PrP (Prnp<sup>−/−</sup>) are totally resistant to the disease.
[30], although it is not impossible that the protein is an intermediate yet indispensable molecule merely mediating neuropathology [18].

2. The immune system in prion diseases

A long incubation period preceding the neurological symptoms is common to all TSEs. Much evidence indicates that TSEA replication as described above is essential for the development of clinical disease and that such replication occurs, at least primarily, in the immune system.

In spite of the lack of any signs of an immune response, it has been known for a long that the immune system plays a crucial role in TSE pathogenesis [31]. Splenectomy of mice before infection significantly prolongs the incubation period of the disease after peripheral inoculation [32, 33], as does genetic asplenia [34], but athymia and neonatal thymectomy have no effect [33]. Severe combined immunodeficient (SCID) mice lacking mature T and B lymphocytes fail to develop the disease [32], but become susceptible after reconstitution with normal splenocytes [35]. Immunosuppressive drugs can prolong the incubation time of the disease [36], whereas stimulation of the lymphoid systems with mitogens enhances disease susceptibility [37].

Following peripheral infection, PrPSc is detected in the spleen, lymph nodes and other lymphoid tissues long before CNS involvement [38-40]. The spleen however may not be the main organ involved in infection via the oral route, infectivity being found first in the Peyer’s patches [39]. This suggests that the gut-associated lymphoid tissues
(GALT) may serve a similar processing and replicative function for intestinally-routed inocula. Early accumulation of PrP\textsuperscript{Sc} in GALT, including mesenteric nodes, has also been described in sheep with scrapie, a natural TSE infection. At a later stage and to a lesser extent, the agent spreads to other systemic or non-gut-associated lymphoid tissues including the spleen [41-44]. Interestingly splenic PrP\textsuperscript{Sc} accumulation is not detectable in cows naturally infected with BSE [45], but is evident in experimental animal models including BSE-infected mice [46] and sheep [47].

Thus peripheral lymphoid organs are important and perhaps indispensable in the transmission and development of the neurodegenerative disease. It is now believed that in most infectious forms of TSE, not only must the infectious agent transit through, but also replicate in the lymphoreticular compartment before invading the central nervous system [31]. Kaeser and colleagues showed recently that optimal prion replication requires PrP\textsuperscript{C} expression by both stromal and haematopoietic compartments [48]. The questions then become: (1) what cell type(s) in the lymphoid organs that may support replication and propagation of prions; and (2) how exactly do these infectious proteins travel from their sites of penetration (e.g. gut) to sites of replication (lymphoid organs) thence into the CNS. A good understanding of these processes is obviously very important in early diagnosis as well as the development of new strategies for treatment and even prevention of the disease.

3. Follicular dendritic cells as the site for prion propagation
The cell type(s) likely to be involved in propagation of prions are thought to be of low density [49], long-lived and mitotically quiescent cells [50]. Follicular dendritic cells (FDCs) in the germinal centres of lymphoid organs or tissues display all these characteristics, and they are also strong PrP-expressing cells [51]. They have been long suspected to be the main cell type targeted by the TSE agents. In studies by many different groups, FDCs [31, 43, 51-53] as well as some macrophages or macrophage-like cells [41, 43, 54, 55] are reported to be sites of PrPSc accumulation soon after peripheral infection. It has been suggested that FDCs can capture prions through their complement receptors [56, 57], a similar cellular interaction described previously for the retention of HIV viruses by the cells [58]. The cell-free conversion of PrPC to PrPSc in the presence of PrPSc [59] suggests it is also possible that PrP may act as an autoligand.

Based on findings from several genetically modified mouse models, however, other groups of investigators argued that B cells [60], and possibly other unidentified cells [61], might be also crucial in peripheral prion transmission. The authors demonstrated that mice devoid of B, but not T, cells were resistant to disease inoculation via peripheral routes, while the animals remained equally sensitive to infection by intracerebral injection [62]. The same group reported subsequently that PrP expression in B lymphocytes was not required for prion neuroinvasion, since immunological constitution with haematopoietic precursors from PrP knock-out donors restored sensitivity to disease induction [60]. One explanation may be that B cells are not directly involved in replication but rather transport of the agents. Alternatively, the lack of B cells or B cell products may prevent FDC maturation and differentiation [63, 64]. By using chimeric mouse models mismatched in PrP status
between FDCs and other cells of the immune system, Brown and colleagues showed that replication of prions in the spleen depends on PrP-expressing FDCs rather than on lymphocytes or other bone marrow-derived cells, and that the contribution of B cells is related to their ability to induce the functional maturation of FDCs [65]. The role of FDCs in TSE pathogenesis, at least acting as the primary sites for prion replication, is once again emphasized and largely confirmed.

4. Entry and transport of orally-acquired TSEA

An important question is how do FDCs acquire TSE agents following intestinal delivery, since FDCs are resident cells in B-cell follicles of organized lymphoid tissues (i.e. not peripheral tissues). TSE agents must first cross the intestinal epithelial barrier and be transported via lymphatics to the draining mesenteric nodes, or via the blood stream to the spleen (which lacks afferent lymphatic drainage). Transport of whole proteins or particles across intact epithelium is very inefficient. Thus it seems unlikely that passive transport across the epithelium and random distribution via the blood or lymph would suffice to deliver sufficient infective material to the lymphoid organs to initiate an infection.

Intestinal epithelium consists of a continuous sheet with individual cells joined by tight junctions. It forms an effective barrier to the movement of molecules and particles into the underlying connective tissues. The seal is however by no means complete, and it is possible that small amounts of macromolecules do transverse the epithelial barrier by transcytosis. In Peyer’s patches and intestinal lymphoid follicles, the overlying epithelium is specialized to facilitate the transport of macromolecules
and particles, and their delivery to lymphocytes and antigen presenting cells (APC) including DC. This transport is mediated by M cells, specialized epithelial cells that have poorly developed microvilli and that are able to rapidly transcytose tracers such as horse-radish peroxidase and fluorescent latex particles to the subjacent areas of the patch [66]. The ability of M cells to transport particles and macromolecules has been ‘hi-jacked’ by a number of intestinal pathogens to enable them to across the epithelia barrier [67-72].

5. Migrating intestinal dendritic cells transport PrPSc from the gut

It is important not to confuse follicular DC with the ‘conventional’ DC also known as the ‘Steinman’ DC [73, 74]. As compared in Table 1, the two types of cells share almost nothing except their ‘dendritic’ morphology. FDCs are long-lived cells that retain immune complexes on their surface for years and present them to B cells [75]. They are probably not haematopoietic. In contrast the ‘Steinman’ DC is a short-lived bone-marrow-derived cell found both in peripheral tissues and in the T cell areas of lymphoid organs. In peripheral tissues, they are actively endocytic [76] and in some circumstances are macropinocytotic, facilitating uptake of large amount of solutes and small particles [77]. The main function of these cells is to acquire antigens in peripheral tissues and transport them, via draining lymphatics, to secondary lymphoid organs [78]. DCs present processed antigen, as small peptides in association with MHC molecules, primarily to T cells, although they can also present intact antigens to B cells [79]. Some DCs are found to express the normal PrP protein [80, 81]. Importantly, unlike FDCs, DCs are actively migratory.
Intestinal DCs are bone marrow-derived cells of the ‘conventional’ DC type, which migrate from the intestinal wall to draining mesenteric nodes. The area of PP underlying the dome is rich in DCs, macrophages and lymphocytes [82, 83]. DCs have also been described in the lamina propria (LP) [84]. Unlike tissue macrophages [85], DCs spend only a short time (2-4 days) in the intestinal wall, and migrate continuously to mesenteric lymph nodes via lymph. They have been shown to carry antigens acquired from the intestinal lumen [86], and apoptotic enterocytes [87]. These migrating cells are therefore in a unique position to transport prions from the gut. Andreoletti and colleagues have shown that in naturally infected sheep, PrP\textsuperscript{Sc} deposits are found in association with a population of CD68-positive cells in the dome area and B follicles of PP before being detected in FDCs [43]. CD68 is a macrophage marker but it is also expressed at low level on some DCs [88, 89]. By using thoracic duct cannulation of mesenteric lymphadenectomized rats, we have shown that intestinal DC but not T or B lymphocytes could indeed acquire PrP\textsuperscript{Sc} from the intestinal lumen and transport them in lymph towards the mesenteric nodes within hours of oral infection (Fig.1) [90].

It is yet to be determined if DCs acquire PrP\textsuperscript{Sc} after it has been internalised by M cells, or by direct uptake across the mucosal epithelium, as recently shown for the transport of apoptotic intestinal epithelial cells [87] and bacteria [91]. At least some DCs are known to leave PP and arrive in mesenteric nodes, and Szakal and Tew have previously shown a cell population in the cortex of lymph nodes which can transport immune complexes from the subcapsular sinus, where DCs are frequently found, to FDCs [92]. Whether and how DCs release prions for uptake by FDCs remains unknown, but DCs can release intact antigen in vivo for recognition by B cells.
Release of cellular contents after cell death (DCs normally die within 3 days after reaching lymph nodes) is another possibility. It is of course also possible that oral TSE agents, once transported to ileal PP, probably via M cells, can reach and replicate on FDCs in situ, subsequently travelling to the CNS via enteric nerve endings [54, 93-95]. However we have shown that they can also travel quickly to mesenteric nodes by hijacking intestinal DCs as a Trojan horse [90].

6. Natural barriers for oral prion entry?

In the experiment described above in which we could show PrPSc in DCs after intestinal delivery, we were unable to show that they contained infective material [90]. This probably reflects the sensitivity of the assay given that only a small number of cells could be injected into each mouse used in the assay, and of those DCs injected, only a small proportion (usually 0.5-5%) had acquired detectable amounts of PrPSc. A rough estimate suggests that only about one in ten thousand molecules given intestinally can be recovered from the DCs travelling in the thoracic duct lymph over a period of 16 hours. The efficiency of oral infection in animal models is generally very low in that ten times as much agent needs to be given orally compared to direct intra-cerebral inoculation [10]. Aucouturier and colleagues have demonstrated, using RAG knockout mice, that infected CD11c+ splenic dendritic cells (conventional DCs) alone, injected systemically (i.v.), are sufficient for prion propagation and transmission to the CNS [96]. The mice developed clinical disease without accumulation of prions in the spleen. This implies that CD11c+ dendritic cells can transport prions from the periphery to the CNS by a route not involving any additional lymphoid element.
An alternative explanation for the low efficiency of oral infection is that there may exist strong natural barriers for the pathogen in the gut, including possibly the gastric-intestinal enzymes, and the cellular enzymatic activities in DCs. As shown in Figure 1, the PrPSc detected in the DC lysate appears to have been modified in some way and the characteristic three-band molecular signature after PK-treatment is not seen. This may be due, at least in part, to antigen processing in the DCs, since we have also demonstrated in vitro uptake of prion proteins by DCs leads to denaturation of the protein, followed by a time-dependent reduction in PrPSc detectable by immunoblotting (Fig.2) [90, 97]. This might sound surprising, as PrPSc is widely regarded as a protease-resistant molecule. The PrPSc fingerprints that identify prion strains are judged by the resistance of PrPSc to Protease K (PK), although its relative resistance to other proteases such as trypsin has also been demonstrated [98]. PK is however a fungal enzyme extracted from a mould (Tritirachium alnum). The enzymes in the cellular compartment and particularly gastro-intestinal tract are complex and identification of those responsible for PrPSc degradation may be of therapeutic significance. It is also essential of course to determine whether the reduced immuno-reactivity detected in DCs also correlates with diminished infectivity as previously reported for macrophages [99, 100].

These findings suggest that apart from the physical barrier formed by intestinal epithelium mentioned above, intracellular and possibly gastric-intestinal enzymes may be able to degrade prions – providing a natural barrier for prion entry that seems to have been largely overlooked previously. This barrier may explain the relative inefficiency often observed for oral/intragastric inoculation with TSE agents [10]. It
may also explain why despite many people in the UK consuming infected beef only a very limited number of people have so far developed nvCJD and why a decline in nvCJD cases [101] rather than an epidemic as previously predicted [102, 103] appears to be the current trend. Nevertheless, whereas transmission of BSE to human (causing nvCJD), and of scrapie to laboratory animals, via the oral route seems to have been relatively inefficient, transmission of BSE to cattle appears to have occurred much more readily, as this was the mode of transmission that led to the epidemic of BSE. Furthermore, as mentioned above, splenic PrPSc accumulation or replication does not appear to be necessary in the BSE-cattle transmission. An interesting and important question one may ask is therefore does this imply that cattle are somehow lacking in such degradative enzymes.

**7. Inflammation - “danger” in another way?**

The conclusion to be drawn from above findings is that the transmission of prion disease, especially via the oral route, may depend critically on a balance between clearance and propagation of the agent in the host. The normal intestinal immune system displays a balance in which pro-inflammatory and anti-inflammatory cells and molecules are carefully regulated to promote immunity against harmful invading pathogens while avoiding responses to self tissues and harmless dietary components [104]. The lack of inflammatory response in oral TSE infection may prevent the activation of macrophages whose phagocytic, enzymatic as well as phago-lysosomal fusion activities can be up-regulated by inflammation, speeding up the clearance process of the agent. It has been shown recently that the prion protein fragment 106-126 is a chemoattractant for monocyte-derived immature but not mature DCs [105,
106], and on which the receptor has also been identified [105]. This might mean that the initial migration of immature DCs towards PrP Sc can be regulated by the agent itself in the absence of overt inflammation. On the other hand the prion fragment can also enhance subsequent monocyte production of proinflammatory cytokines [105]. Inflammation stimulates the migration and maturation of DCs, which facilitates the transport of prion, and it is known that antigen processing is down-regulated in mature DC. The effects of inflammation may therefore alter the balance of prion uptake, retention and transport by DCs in several different ways, depending possibly on the stages of their transmission, DC maturation, and perhaps selectivity in the inflammatory mediators involved. It would make sense that the observed effects of immunosuppressive drugs on TSE susceptibility [36] could be due to their actions on the inflammatory machinery rather than the specific (B/T cells) immune system [107].

8. Therapeutic and preventive prospects

As described above recent evidence has indicated a role of DCs in delivering and possibly propagating prions following oral inoculation. One should remember however that the main function of DC is to initiate immune responses. Prion-infected animals can develop normal immune responses, both humoral and cellular, to conventional antigens or mitogens [108, 109] but the absence of immune reaction to PrP Sc is not surprising as it differs from the PrP C molecule only in tertiary structure. The fact that PrP C is a host-encoded protein and the immune system is presumably already tolerized to the molecule explains the lack of response.
However it is quite possible that although T cells are tolerized, B cells are not. This is because the mode of antigen recognition by T and B cells is quite distinct. T cells can recognize only processed antigen as peptides in the context of MHC molecules. Since PrP\textsuperscript{Sc} and PrP\textsuperscript{C} share the primary structure (amino acid sequence, Diagram A), in theory, no T cell response would be expected, as reactive T cells would have been deleted in the thymus or tolerized in the periphery. However, the conformational changes of the protein may create new (foreign) epitopes (Diagram B) for B cells, which recognize native, unprocessed antigen. This implies that a B cell response may be potentially inducible providing that alternative T cell help can be offered. Encouraging findings have recently suggested a role for anti-prion molecules in disease prevention in a transgenic (mu chain anti-PrP) mouse model (Prnp knockout) [110]. This could provide an opportunity and rationale for novel therapeutic strategies in vaccine design by delivery of synthetic peptides with purpose-built T cell epitopes (Diagram C) attached to known B cell epitopes [111] leading to T-B, or DC-T-B (see below and Diagram D), collaborative responses. Efforts to identify such new B and T cell epitopes will rely on a good understanding of the 3-D structure not only for PrP\textsuperscript{C} [112] but, more importantly, for PrP\textsuperscript{Sc}, yet to be obtained.

Finally, DCs are a heterogeneous population of cells. DCs with immunogenic and tolerogenic properties have been shown in human and a variety of animal models [113, 114]. In addition some DC subpopulations can retain intact antigen more efficiently than others. Identification and characterization of particular DC subsets that are responsible for transmitting the disease, that are mediating immunity against the causal agents, and their responses to inflammatory stimuli may also prove to be informative. Although the transport of prions by DC is an early event of the infection
and hence is not likely to be a suitable site for intervention, potential therapeutic approaches may still be focused on how specific immunity to the agents can be effectively induced. These professional APC can be employed as a vector to deliver the ‘modeled vaccine’ mentioned above (Diagram D). In summary, a better understanding of all these aspects in prion pathogenesis could lead to rational immuno-manipulation strategies aimed at preventing the replication and spread of the infectious agents to the CNS.
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System - Dendritic Cells, Immune Regulation and Tolerance, Current
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<td>Bone marrow-derived, haemopoietic</td>
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<td><strong>Distribution:</strong></td>
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<td>Peripheral tissues, secondary</td>
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<tr>
<td></td>
<td>(B cell follicles)</td>
<td>lymphoid organs (T cell areas)</td>
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<tr>
<td><strong>Life-span:</strong></td>
<td>Long (years)</td>
<td>Short (days - not Langerhans cells)</td>
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<td>Antigen presentation (B cells - as immune complexes)</td>
<td>Antigen presentation (peptides to T cells on MHC)</td>
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<td>Actively migratory</td>
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**Figure 1.** Intestinal DCs transport PrPSc to mesenteric nodes via lymph. Lymph was collected 8 to 16 h after intestinal injection of mouse scrapie-associated fibrils (ME7 SAF). Conspicuous PrP+ cytoplasmic inclusions were detected by immunocytochemistry in a small proportion of DCs (a) but not B (b) or T (c) lymphocytes in the thoracic duct lymph of mesenteric lymphadenectomized rats. (d) Control DCs from PBS-injected animals. Magnification x1000.

(e) Immunoblot analysis of pooled cell lysates (1x10^6 cells per lane) from SAF-treated rats confirmed the presence of PrPSc in lymph DCs (lane 4) but not in T or B lymphocytes. SAF equivalent to 2 or 4 µg of infected brain tissue was loaded in lanes 5 and 6, respectively. Treatment of samples in the presence (+) or absence (-) of proteinase K (PK) before electrophoresis is indicated. In all panels, PrP was detected using the PrP-specific polyclonal antiserum 1B3.

*(Huang et al. JGV 2002; 83:267-271)*

**Figure 2.** DCs acquire and process PrPSc following in vitro culture with SAF.

Bone marrow-derived DCs (BMDC, 1x10^6 cells) were cultured in the absence (BMDC alone) or presence of SAF (equivalent to 10 mg infected brain tissue) for the times indicated. Immunoblots show the accumulation of detergent-insoluble, relatively proteinase K-resistant PrPSc within BMDC lysates. Treatment of lysates in the presence (+) or absence (-) of proteinase K (Pk) is indicated. SAF was incubated in medium alone as a control. Following Pk treatment, a typical three-band pattern was observed between molecular mass values of 20 and 30 kDa, representing unglycosylated, monoglycosylated and diglycosylated isomers of PrP (in order of
increasing molecular mass). SAF equivalent to 50 µg infected brain tissue and/or BMDCs equivalent to 104 cells were loaded per lane.

(Huang et al. JGV 2002; 83:267-271)

**Figure 3: Modeling T cell epitopes for inducing DC-T-B cell collaborative responses against PrPsc.** Schematic representations of the primary sequence (A) and tertiary structure (B) of PrPsc, and the purpose-built synthetic new epitope for T cell recognition (C). Cellular interactions, antigen presentation and recognition by DC, B and T cells after encountering the natural B cell epitope (b d f) and the synthetic T (and B) cell epitope (b’d’f’) are depicted in (D).