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T cell memory specific for self and non-self antigens in rats persistently infected with Borna disease virus

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SUMMARY

We have studied CD4+ Th1 T cell responses in Borna disease (BD), a virus-mediated immune disease of the central nervous system (CNS), and demonstrate the priming of virus-specific as well as autoreactive T cells specific for myelin antigens in the course of viral infection. The fate of these *in vivo* generated T cells was subsequently assessed by *in vitro* proliferation assays with lymphocytes from different lymphoid organs of diseased animals over a long period of time. Virus-specific T cell responses continuously decreased during the establishment of persistent infection and could no longer be detected after 5–6 months *post infectionem*, when inflammatory reactions in the brain had ceased. By contrast, autoantigen-specific T cells kept their ability to mount characteristic secondary responses—although at an overall rather low level—over long periods of time; these autoreactive T cells homed to a specific lymphoid organ, the perithymic lymph node. Our study thus describes for the first time a complete decline of virus-specific T cell memory in a persistent viral infection, and raises the question how long-lasting T cell autoreactivity is controlled.

Keywords Borna disease virus autoreactivity T cell memory T cell homing viral persistence

INTRODUCTION

This study addresses the question of T cell memory acquired during a viral infection, illustrated in Borna disease of Lewis rats. Borna disease is an immune-mediated, virus-induced neurological disorder characterized by profound behavioural disturbances of infected animals [1,2]. The disease is caused by a strictly cell-associated and non-cytopathic virus that has recently been classified as an RNA virus [3,4]. In adult animals the virus is found exclusively in central nervous system (CNS)-derived cells, mainly in neurons and to a lesser degree in astrocytes and ependymal cells, both in the acute inflammatory phase as well as during the non-inflammatory persistent chronic phase of the disease. This phase of viral persistence is characterized by continuing neurological symptoms, accompanied by high anti-viral antibody titres, but surprisingly a complete cessation of cellular infiltrations in the CNS. The reasons for this discrepancy between a vigorous initial anti-viral cellular immune response and the subsequent drastic waning of inflammation, despite continued antigen expression in the brain, remain an enigma [1].

The immune-mediated character of Borna disease has been determined by transfer experiments with immune cells, particularly with a virus-specific CD4+ T cell line into healthy Borna disease virus (BDV)-carrier recipients, which subsequently develop the typical signs of disease, such as ataxia, fearfulness and hyperactivity [2,5]. Immunohistological studies of brain lesions, which have revealed a predominance of CD4+ T cells, macrophages and late B cells [6], are further suggesting that BD is a consequence of a DTH reaction in the brain.

Autoimmune responses against myelin components have been documented in different animal models of virus-induced CNS diseases. Examples comprise coronavirus-induced encephalomyelitis of Lewis rats [7], characterized by marked demyelinating plaques and high titres of infectious virus, or measles-induced subacute encephalomyelitis in rats [8], where signs of demyelination can hardly be observed. The contributions of these autoimmune reactions against myelin components to the respective immunopathology remain therefore to be determined. Furthermore, the exact mechanisms responsible for initiating autoimmunity have not been elucidated in any of the various models of virus-induced encephalitis.

In the present study, we investigated T cell responses to viral antigens and CNS autoantigens in the course of BDV infection. CD4+ T lymphocyte responses specific for viral antigens and two different self-antigens, myelin basic protein (MBP) and proteolipid protein (PLP), were detected. We subsequently focused our interest on the fate of these *in vivo* generated T cells in terms of homing pattern and the maintenance of long-term responsiveness, i.e. T cell memory. T cell responsiveness was tested throughout a 6-month observation period of viral persistence and analysed in different primary and secondary

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lymphoid organs, namely thymus, spleen, perithymic and mesenteric lymph nodes.

MATERIALS AND METHODS

Virus and animals

The Giessen strain He/80 of BDV was used throughout this study [6,9]. Four-to-five week old female Lewis rats were obtained from the Zentralinstitut für Versuchstierzucht (Hannover, Germany), and were infected via intranasal route with 6×10^4 ID_{50} of the virus.

Antigens

BDV-antigen (38/39 kD and 24 kD proteins) were extracted from a persistently BDV-infected MDCK cell line and purified by affinity chromatography as described previously [10]. MDCK extracts were used as controls to determine the specificity of anti-BDV T cell responses. MBP was prepared from Lewis rat brain as described previously [11]; ovalbumin (OA) and concanavalin A (ConA) were from Sigma (St Louis, MO). For evaluation of PLP responses we used a synthetic peptide of murine PLP [12] corresponding to amino acids 130–150 (QAHSLERVCHCLGKWLGHPDKF), which was synthesized using an automatic peptide synthesizer (Applied Biosystems) according to standard protocols. MBP- and PLP-antigen preparations were tested for the determination of optimal antigen concentrations with long-term antigen-specific rat T cell lines.

Clinical evaluation

Animals were examined daily and weighed, and disease severity was scored on an arbitrary scale ranging from 0 to 3 (grade 0, no symptoms; grade 1, slight incoordination and fearfulness; grade 2, distinct ataxia or slight paresis; grade 3, paresis or paralysis).

Proliferation assays and cultivation of T cell lines

Splenocytes (2×10^5) from infected animals were cultured in round-bottomed 96-well microplates in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), 1 mM L-glutamine, 10 mM HEPES, 5×10^{-5} M 2-mercaptoethanol (2-ME), penicillin and streptomycin. Cells were incubated in the presence of different antigens for 96 h at 37°C and 5% CO_2, and ^{3}H-thymidine was added for the following 6 h before collecting; proliferation was assessed by standard liquid scintillation techniques. S.E.M. was usually 10%. Stimulation index (SI) is defined as ct/min of antigen-stimulated cell cultures ÷ ct/min of unstimulated cultures. For establishment of T cell lines, cells were restimulated every 8–9 days in the presence of irradiated syngeneic thymocytes ($2{\cdot}5 \times 10^7$/ml) and the specific antigen. After restimulation, T cell blasts were separated by Ficoll–Hypaque density gradient centrifugation and then cultured in the presence of 10% Con A supernatant.

Antibody and cytokine detection

Antibody production was determined by standard ELISA. Polystyrene flat-bottomed plates were first coated with poly L-lysine (100 μg/well) overnight at 4°C. After washing with 0·05% Tween in PBS and then with water, the plates were coated with MBP (10 μg/ml) overnight at 4°C. After blocking with 3% bovine serum albumin (BSA) in PBS for 1 h at 37°C and then 1 h at room temperature, serum samples or graded amounts of control antibodies were added overnight at 4°C. After washing, bound IgG was detected by adding peroxidase-labelled goat anti-rat IgG (Jackson Immunoresearch) for 1 h at room temperature. ABTS substrate (Boehringer Mannheim Biochemica) was added to the plates for 5 min and colour development was read at 405 nm wavelength in a Titertek Multiskan (Flow Labs). As positive control, serum from rats immunized with MBP-Freund's complete adjuvant (FCA) (100 μg MBP/animal) was used.

For measuring the amount of interferon-gamma (IFN-γ) in supernatants from stimulated T cell cultures, a cytokine-specific ELISA was employed as previously described [13]. Briefly, ELISA plates were coated with 100 μl of 5 μg/ml coating antibody in $NaHCO_3$ buffer (pH 8) and incubated overnight at 4°C. Wells were blocked, washed extensively, and samples and standard dilutions of recombinant rat IFN-γ were added in triplicate. After a 4-h incubation at room temperature, peroxidase-conjugated antibody against hIFN-γ (300 pg/ml), which cross-reacts with rat IFN-γ, was added and colour development was performed as described above.

IL-2 secretion was tested by proliferation assays with the IL-2-dependent murine cell line CTLL. CTLL line cells (2×10^3) were incubated for 24 h with 100 μl of sample supernatants, and proliferation was determined by measuring ^{3}H-thymidine incorporation for the following 12 h; data were quantified with the help of a recombinant IL-2 standard curve.

Statistical analysis

Student's *t*-test was used to compare mean ct/min scores of responder T lymphocytes for a certain antigen in infected and non-infected animals.

RESULTS

Myelin protein-specific T cell responses

Lewis rats were infected with the Giessen strain He/80 of BDV by intranasal application, which most closely mimics the naturally occurring infection route [14]. After the onset of the acute phase of disease, characterized by ataxia, fearfulness, hyperactivity and sometimes paralysis, the animals were killed at various intervals and single-cell suspensions from spleens were tested in primary restimulation assays for responsiveness to virus proteins and myelin proteins. Peripheral lymphocytes isolated from animals 25 days *post infectionem* displayed a detectable proliferative response against not only viral proteins, but also against MBP and PLP. This self lymphoproliferation was significant ($P < 0{\cdot}01$) compared with that obtained with lymphocytes isolated from uninfected animals. Reactivity against myelin components could be observed in 10/17 animals tested during the acute phase of disease, as shown in Table 1, Group 1. Group 2 of Table 1 shows the proliferative response of splenocytes isolated from three infected animals, where a significant proliferative response was detected only for viral antigens. Group 3 of Table 1 presents, as full negative control, a set of proliferative responses of splenocytes from three non-infected animals. SI were in the range of 1·9–4·4, which is low when compared with BDV-specific SI. However, ranges of autoreactive T cell responsiveness in BDV-encephalomyelitis are comparable to those described in other virus-mediated CNS diseases, e.g. coronavirus-induced encephalitis [7] or subacute measles encephalomyelitis [8].

Table 1. T cell responses during acute Borna disease (BD) encephalomyelitis (25 days after infection)

	^{3}H-thymidine incorporation (ct/min)					
Animal no.	No antigen	MBP	PLP	BDV	OA	Con A
Group 1, infected rats						
1	1209	2334	2653	8311	1154	38711
2	926	2311	2898	6443	832	34091
3	2099	4209	5231	10741	1886	45406
4	971	1943	3231	8187	1021	28445
5	1228	2961	3704	7712	941	32981
6	953	1772	2429	7831	897	32773
7	1325	2743	3289	5914	1301	36080
8	843	2031	2661	7231	722	30974
9	1016	2519	3899	9021	887	42117
10	1565	3553	4451	12372	1345	43907
Group 2, infected rats						
11	964	1053	1275	6068	873	32214
12	1369	1232	1376	7396	1154	44387
13	1117	913	1059	8216	1098	38774
Group 3, non-infected rats						
1	1381	947	1204	1277	1265	33651
2	1047	564	1006	1131	983	35209
3	1245	612	1087	1241	1042	34277

BDV-infected animals were killed at day 25 after infection. Splenocytes (2×10^5) were cultivated in 96-well round-bottomed culture plates in the presence of rat myelin basic protein (MBP; 20 μg/ml), proteolipid protein (PLP; 10 μg/ml), BDV-antigen (40 μg/ml), ovalbumin (OA; 40 μg/ml) or concanavalin A (Con A; 1 μg/ml). Cultures were pulsed with ^{3}H-thymidine from 96 to 102 h for determination of antigen-specific responses and from 72 to 78 h for mitogen-driven activation. Results represent mean proliferation of triplicates, with s.d. always < 15%.

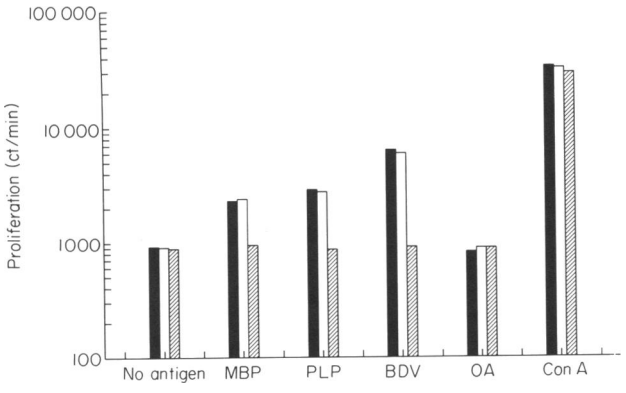

Fig. 1. MHC restriction of T cell responses. Splenocytes from infected rats were cultivated in 96-well round-bottomed culture plates at 2×10^5 cells/well in the presence of different antigens (for details see Table 1). Proliferation was assessed in the presence or absence of MoAbs (10 μg/ml) specific for MHC class I (OX18) or class II (OX6) determinants. ■, No antibody; □, anti-MHC class I; ▩, anti-MHC class II. MBP, Myelin basic protein; PLP, proteolipid protein; BDV, Borna disease virus; OA, ovalbumin; Con A, concanavalin A.

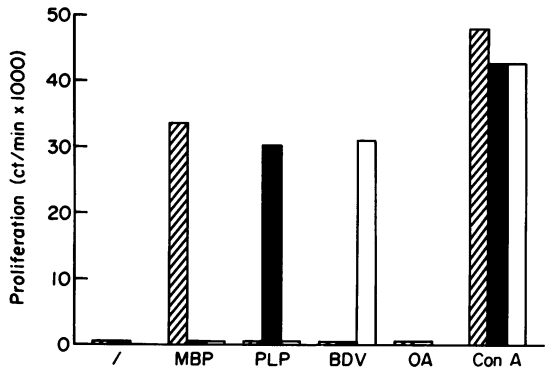

Fig. 2. Virus-specific and autoantigen-specific T cells display no cross-proliferative responses. Splenocytes from acute infected animals were stimulated in the presence of the respective antigen and T cell blasts separated by Ficoll gradient centrifugation. T cells were subsequently stimulated for two restimulation rounds *in vitro* and then tested for their potential cross-reactivity for the other two antigens. Resting T cells (1×10^4) were incubated with 3×10^5 irradiated syngeneic thymocytes as presenters and antigen (myelin basic protein (MBP) 20 μg/ml; proteolipid protein (PLP) 10 μg/ml; Borna disease virus (BDV) 40 μg/ml; ovalbumin (OA) 40 μg/ml) or mitogen (Concanavalin A (Con A 1 μg/ml). Proliferation was measured by the incorporation of ^{3}H-thymidine during the last 16 h of a 3-day incubation period. Data were obtained in triplicate cultures with s.e.m. < 10%. □, BDV-specific T cells; ■, PLP-specific T cells; ▩, MBP-specific T cells.

Phenotypical characterization

Peripheral lymphocytes from acute infected animals were antigen-stimulated in the presence or absence of MoAbs specific for MHC class I or class II determinants of rat. As depicted in Fig. 1, proliferative responses against viral and auto-antigens could be suppressed by class II-specific antibodies (OX6), but not by antibodies directed against class I (OX18). Next, we analysed T cells from diseased rats for their phenotype. T cells isolated from infected animals (day 25 p.i.) were propagated *in vitro* for two restimulation cycles in the presence of the respective antigen. MBP-, PLP- and BDV-specific T cell lines displayed a cell surface expression characteristic for CD4$^+$ T cells (data not shown). Supernatants from stimulated cells could strongly activate the IL-2-dependent murine CTLL-2 cell line and contained high amounts of IFN-γ (5500 pg/ml IFN-γ in stimulated cultures compared with < 100 pg/ml in unstimulated cultures). On this basis, the effector cells were categorized as corresponding to the Th1 subset of CD4$^+$ T cells, as originally defined in mice. Based upon recent reports [15,16] it is likely that Th1 and Th2 subsets exist likewise in rats.

Testing for cross-reactivity

Among the possible mechanisms responsible for initiating immune responses and maintaining T cell memory, cross-reactivity is clearly one of the favourite candidates [17]. When T cells obtained from BDV-infected animals were tested, cells specific for myelin antigens did not cross-react with viral antigen, nor did the BDV-primed T cell line show a proliferative response to the two self-antigens, MBP and PLP (Fig. 2). We conclude that cross-reactivity between viral and myelin antigens probably plays no significant role in BDV-triggered generation of autoreactive T cell responses.

Table 2. Long-term T cell responsiveness in different lymphoid organs

Months after infection (clinical grade)	Organ	Antigen					
		No antigen	MBP	PLP	BDV	OA	Con A
1 (3·0)	Thymus	818	544	712	1544	810	30662
	Spleen	2097	3367	4589	14889	1886	45003
	pt-LN	1998	2199	2322	3009	1944	43912
	mes-LN	907	821	815	1441	812	27702
1·5 (1·5)	Thymus	596	351	390	4493	412	33466
	Spleen	2571	4922	5009	30567	2411	51725
	pt-LN	1104	2257	2782	3523	896	42926
	mes-LN	1531	1045	1488	3955	1711	37544
2 (1·5)	Thymis	651	435	608	4127	578	24101
	Spleen	2513	1736	2419	23352	2210	35133
	pt-LN	1081	1813	2722	2102	795	26161
	mes-LN	1453	1123	1390	4173	1410	29129
2·5 (0·5)	Thymus	881	531	491	2591	538	37001
	Spleen	3113	2307	2664	26817	1788	54790
	pt-LN	2173	3812	5122	2023	2444	39076
	mes-LN	1661	1530	1515	2009	1613	42036
4 (0·5)	Thymus	432	312	349	556	612	28213
	Spleen	1546	1433	1077	3988	1433	49774
	pt-LN	848	2095	1564	759	811	30554
	mes-LN	2109	1415	1806	1844	1676	36651
5 (0·5)	Thymus	481	271	344	327	404	26150
	Spleen	1573	2199	3358	1903	1333	41087
	pt-LN	1334	2811	3189	1090	1211	32274
	mes-LN	ND	ND	ND	ND	ND	ND
6 (0·5)	Thymus	ND	ND	ND	ND	ND	ND
	Spleen	2048	2201	2498	1887	1917	39065
	pt-LN	753	1906	2832	633	814	22076
	mes-LN	ND	ND	ND	ND	ND	ND

Lymphocytes of different lymphoid organs (thymus, 5×10^5 cells/well; spleen, 2×10^5 cells/well; perithymic lymph nodes (pt-LN) and mesenteric lymph nodes (mes-LN), 2×10^5 cells/well) were cultivated in the presence of different antigens in triplicate cultures. Proliferation was determined by addition of ^{3}H-thymidine from 100 h to 106 h for assessment of antigen-specific responses and from 72 h to 78 h for assessment of mitogen-driven stimulation. Each determination is representative of a group of three animals. Differences in response to myelin basic protein (MBP), proteolipid protein (PLP) or Borna disease virus (BDV) antigens statistically significant are underlined ($P < 0{\cdot}01$). Statistically significant autoreactivity in the pt-LN was found in 5/7 infected animals at 2·5 months after infection, 4/7 animals after 4 months, 5/7 animals after 5 months, and 4/7 animals after 6 months (0/10 animals in non-infected rats). BDV-specific T cell responses were found in 7/7 animals 2·5 months after infection, 7/7 animals (4 months), 1/7 animals (5 months), 0/7 animals (6 months), and in no animal of the non-infected control group.

Antibody responses

Similar to cellular-mediated autoreactivity, antibodies with host-derived neuronal antigens have been described in different models of virus-triggered CNS diseases [8,18,19]. To determine a potential humoral response to MBP in Borna disease, sera of infected rats were tested in an enzyme-linked immunoassay. During the acute phase of encephalomyelitis low levels of circulating anti-MBP antibodies could be detected in 16/25 independent samples (data not shown). Positive samples were mainly found in infected animals showing an anti-self T cell reactivity. This notion supports the assumption that antigen-specific B cells might be crucially involved in the stimulation of class II-restricted T cells [20,21]. However, when sera of infected animals were re-checked 2 months after overcoming the acute phase of disease, humoral responses were no longer detectable with the readout used. Due to the lack of a full length PLP antigen probe, we could not evaluate the humoral response to this antigen. However, as cellular responses to MBP and PLP always paralleled each other, we would not expect a great difference in the characteristics of antibody responses.

Maintenance of T cell memory

We then tried to evaluate the maintenance or decay of memory responses to self-antigens *versus* a persisting viral antigen. To test memory in CD4$^+$ Th1 T cells, we chose the *in vitro* proliferation assay to the specific antigen, where unprimed cells are unable to mount a response. Furthermore, we followed the migration pattern of these memory cells by checking the long-term survival of responder cells in the thymus and secondary lymphoid organs (spleen, mesenteric lymph nodes (mes-LN),

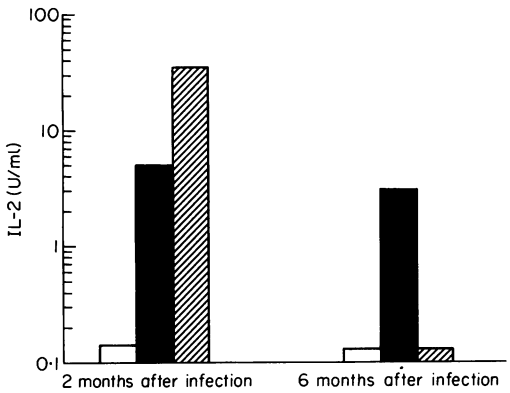

Fig. 3. Determination of T cell memory reactivity by measuring IL-2 release of T cell responses of Borna disease virus (BDV)-infected rats at various times after infection. Proteolipid protein (PLP)-specific T cell responses of perithymic lymph node (pt-LN)-derived lymphocytes and BDV-specific responses of spleen-derived lymphocytes were tested in proliferation assays as described in Table 2. Culture supernatants were harvested after a 80-h incubation period, and IL-2 production was assayed by a standard IL-2 bioassay (CTLL line assay). Proliferation was referred to a recombinant IL-2 standard curve. □, Medium; ■, PLP; ▨, BDV.

perithymic lymph nodes (pt-LN)). The results are presented in Table 2.

One month after BDV infection, both anti-viral and autoreactive T cell responses displayed a marked proliferation in spleen-derived cells, although BDV-specific T cell responses had not reached the maximal level. Furthermore, thymocytes reacted against viral antigen but not against myelin proteins. In the mes-LN and pt-LN no significant reactivity against the autoantigens could be observed, whereas virus-specific reactivities were present, albeit at a low level. Responses to an irrelevant antigen (OA) were not detectable in any of the lymphoid tissues tested.

One-and-a-half months after infection, autoreactive T cell responses to myelin antigens were still measurable in the spleen, and could interestingly be retrieved from pt-LN cells. The anti-BDV T cell response was at its maximum both in spleen- as well as in thymus-derived cells. This picture remained in principle unchanged for the following 4 weeks, when CNS inflammation ceased, despite ongoing disease and virus persistence in the brain (data not shown). At this stage, residual neurological symptoms are rather the consequence of irreversible neuronal damage, and not due to a still ongoing CNS inflammation.

Following the responsiveness pattern over a period of 4 months, we could observe a quite remarkable decline in anti-BDV responses. Whereas anti-BDV reactivity in the spleen could still be demonstrated, although at a rather low level, thymus-derived and LN cells failed to proliferate in the presence of viral antigen. However, autoreactive responses of T cells derived from pt-LN remained pronounced and relatively constant. Autoreactive splenocytes were only very infrequently found at this late stage of disease.

When monitoring the recovering animals for a period of up to 6 months *post infectionem*, we observed a complete decline of virus-specific T cell responses. Five and 6 months after infection, BD-specific reactivity was no longer significantly elevated in peripheral lymphocytes. Interestingly, even at this late stage myelin protein-specific proliferation was demonstrable in lymphocytes derived from the pt-LN. To confirm the results of the proliferative responses, we employed an IL-2 cytokine-detection assay with supernatants from *in vitro* stimulated memory cells (Fig. 3). As in the proliferation assay, autoreactive T cell responses could be demonstrated, whereas viral-specific T cell responses were no longer detectable 6 months after infection.

DISCUSSION

Much of the previous work concerning T cell responses in Borna disease has come from *in vitro* studies with T cell lines derived from immunized, not infected, animals. In contrast, the current study focuses on the homing pattern and maintenance of antigen-specific $CD4^+$ T cell reactivity under conditions mimicking a naturally occurring viral infection. The data obtained by this strategy provide for the first time some evidence that, like $CD8^+$ T cell reactivity [22–24], $CD4^+$ Th1-mediated antiviral memory might be rather short-lived. Furthermore, our experiments imply that an initially vigorous antiviral T cell reactivity can completely wane despite persistence of the immunizing agent, a finding which might be interpreted as a viral escape mechanism from T cell surveillance under antigen-limiting conditions.

No studies have yet been conducted to assess a possible autoimmune component in Borna disease. Our study demonstrates that BDV infection can induce a long-term anti-self T cell response. Autoreactive T cells specific for MBP and PLP, the two major components of myelin proteins, were detected, although at a relatively low level compared with antiviral T cell reactivity. Such a myelin protein-directed self-reactivity of comparable magnitude has already been described in other models of virus-mediated encephalomyelitis in rats, e.g. in coronavirus- [7] and measles virus-induced encephalomyelitis [8].

Autoreactive T cells showed a definite tendency to localize to the pt-LN and remain there for prolonged periods of time. The pt-LN are found in the thymic capsula in rats and in close proximity to the thymus on the capsula in mice [25]. The significance of these lymph nodes in terms of short-term T cell homing has been documented in a recent study, which could demonstrate a selective migration of OA-specific T cells into pt-LN in the absence of exogenous antigen [26]. However, the mechanisms which determine the attraction of activated T cells to this particular lymphatic tissue remain unknown.

How can the decay of anti-BDV T cell memory *versus* the maintenance of self-reactivity be explained? Compelling evidence now emerging suggests that the hallmark for maintenance of T cell memory is the long-term availability of antigen, i.e. the concept of antigen-dependence of T cell memory [22,24,27–29]. The implication of this postulate is that the more poorly replicating a virus is the less long-lasting T cell memory will be. Furthermore, newer data indicate that 'memory' T cells and newly activated effector cells express the same surface molecules and, once activated, can both revert to a phenotype characteristic of naive or resting cells [30,31]. In view of these findings it appears that T cell memory is rather the result of a continuous

antigen-driven activation than due to the presence of specialized, non-dividing 'memory cells' with a prolonged life span.

BDV, unlike certain other CNS-persisting viruses, for example HIV, HTLV-1 and measles virus, shows a definite and strict tropism for neural cells, but is unable to infect blood-derived lymphoid cells. Whereas good evidence exists for BDV antigen persistence, the availability of these antigens to the immune system has not been explored. Interestingly, a recent report from our laboratory could demonstrate that while in the acute phase of inflammation BDV antigen is found both in the nucleus and cytoplasm of infected cells, with the duration of infection the content of antigen is continuously decreasing in the cytoplasm compartment, and can finally only be detected in the nuclei of virus-harbouring cells [6]. Similar examples of limited antigen expression during the course of a persistent viral infection have already been described for other viruses, e.g. lymphocytic choriomeningitis virus (LCMV) infection [32]. Although not directly proven, it is tempting to speculate that this restriction of viral antigen expression in the late phase of BDV infection might be below a certain 'antigenic-driving activation threshold' for maintaining a solid T cell reactivity over time. Our results of waning BDV-specific T cell memory during the chronification of disease extend previous studies, which could show a complete decline in CNS inflammation despite the continuous production of viral antigens in the persistently infected brain in BD rats [1]. It seems that the reason for this decline of CNS inflammation in BD is indeed a corresponding decline of virus-specific T cell memory. Separation from T cell recognition by limiting the availability of viral antigen is therefore the most straightforward explanation for the persistence of BDV infection, despite an initial priming of a vigorous antiviral T cell response. However, further experiments (e.g. *in situ* analysis of BDV messenger expression during persistence) are needed for final confirmation of this hypothesis.

Such a limitation of antigen availability obviously does not hold true for abundantly expressed myelin antigens, such as MBP or PLP. In contrast to the traditional view, fragments of myelin proteins with a high turnover rate are by no means strictly sequestered or 'secluded' within the CNS. More than 10 years ago, Paterson and coworkers were able to demonstrate the presence of circulating MBP fragments in the serum of normal healthy Lewis rats [33], and in both healthy individuals and patients with neurological disorders [34]. This means that fragments of abundantly expressed neuroantigens with a high turnover rate are in principle available to T cell recognition outside the CNS. Consequently, class II-restricted responses to exogenous peptides derived from 'secluded' neuroantigens might be perpetuated for very long periods of time, once immune recognition has occurred. Virus-triggered breakage of tolerance could then be envisaged as the necessary initiating event [35], after which self-reactivity is self-maintained due to the permanent regeneration of circulating immunogenic peptides.

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