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Modulation of acute coronavirus-induced encephalomyelitis in γ-irradiated rats by transfer of naive lymphocyte subsets before infection

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Clinical course, recovery of infectious virus from brain tissue and histopathology of the central nervous system were examined in γ-irradiated Lewis rats reconstituted by naive lymphocytes before infection with coronavirus MHV-4 (strain JHM). Up to 9 days past infection, no differences were seen between immunologically competent and immuno-deficient animals in terms of onset and progression of neurological disease. However, in the latter animals neurological symptoms were dominated by signs of encephalitis instead of paralytic disease as usually seen in immunocompetent animals. Nevertheless, despite high titers of infectious virus in the CNS of immunodeficient animals only mild histopathological changes were noticeable. In contrast, infectious virus in the CNS of immunologically competent animals was below the detection limit of the assay. Paralytic disease and tissue destruction were T lymphocyte mediated because γ-irradiated rats that were reconstituted by CD4$^+$ or CD8$^+$ T lymphocyte enriched cells in the absence of B lymphocytes revealed an earlier onset of clinical symptoms and a more rapid deterioration of their clinical state compared to fully competent animals. Whereas in CD4$^+$ T cell reconsituted animals infectious virus was moderately reduced and tissue destruction as well as inflammatory changes in the CNS were focal, in CD8$^+$ T cell reconstituted animals vacuolizing white matter inflammation was diffuse without reduction of infectious virus in brain tissue. From the presented data we conclude that in the acute stage of JHMV-induced encephalomyelitis of Lewis rats: (i) tissue destruction and paralytic clinical symptomatology are mainly T cell-mediated; (ii) CD4$^+$ T lymphocytes can directly contribute to reduction of viral load in the brain and (iii) only coordinated action of both, the T and the B cell compartment enables animals to survive the infection and recover from disease.

Keywords: coronavirus; central nervous system; encephalomyelitis; lymphocyte subsets

Introduction

Intracerebral infection of Lewis (LEW) rats with Coronavirus JHM (JHMV) is an important animal model to study the complex relationships between viral infection of the central nervous system (CNS), the intracerebral immune system response and focal loss of myelin sheaths (Watanabe *et al*, 1987). The majority of animals (60–70%) infected at the age of 3 weeks develop a neurological disease characterized by ataxic gait, hind leg paresis or tetraplegia. Recovery from this clinical attack is frequent, and occasionally relapsing courses have been reported (Dörries *et al*, 1991; Watanabe *et al*, 1987; Wege *et al*, 1984). In surviving animals, large areas of primary demyelination can be observed throughout the CNS that are typically located close to perivascular cuffs and are infiltrated by numerous mononuclear cells (Watanabe *et al*, 1987). Contribution of individual leukocyte subsets to the cellular exudate as well as their spatial arrangement at virus-infected sites change significantly in the course of the acute disease. The early lymphocyte infiltration is dominated by CD4$^+$ and CD8$^+$ T

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lymphocytes. They constitute more than 90% of lymphocytes homing to virus-infected foci. Kinetic studies have shown that prior to onset of neurological disease the amount of T lymphocytes rapidly increases in CNS tissue to high levels. This level is maintained until animals start to recover from neurological disease (Dörries *et al*, 1991). As published by us, $CD8^+$ T cells isolated from the CNS of JHMV-infected animals exert strong virus-specific MHC class I restricted cytotoxicity *in vitro*. This cytotoxic activity peaks at the same time as the maximum of overt neurological symptoms is reached and lasts until the beginning of recovery (Hein *et al*, 1995). B lymphocytes are always a minority amongst CNS infiltrating lymphocytes, but late after infection a considerable increase of virus-specific antibody secreting plasma cells (AbSC) is notable in the brain parenchyma that regularly coincides with onset of convalescence in the majority of animals (Schwender *et al*, 1991).

These data clearly implicate that histopathological and clinical course of the infection are not exclusively determined by neuropathogenic properties of the virus but are also strongly influenced by cells of the immune system. In this context, it is of interest that 30–40% of immunocompetent LEW rats succumb to an acute encephalomyelitis within the first 2 weeks after infection with JHMV (Dörries *et al*, 1991), suggesting that in these animals the virus-specific immune system response is either delayed or of different quality from animals which survive the infection.

So far, however, the impact of individual lymphocyte subsets on the onset and progression of neurologically overt disease as well as on histological parameters of the infection is unclear. Therefore, we dissected virus-induced neurological deficits from immune system mediated effects in the early stage of infection by examination of clinical course, histopathology and recovery of infectious virus from the CNS in γ-irradiated LEW rats up to 9 days past infection (d.p.i.). Animals were reconstituted by different combinations of enriched naive lymphocyte subsets before intracerebral infection with coronavirus JHM.

Based on the presented and previously published data, we assume that the rather late appearance of virus-specific AbSC in the CNS of immunocompetent LEW rats enables unrestricted extracellular viral spread and formation of multiple secondary virus-infected foci throughout the entire brain and spinal cord during the first week past infection. As a consequence, a vigorous inflammatory T lymphocyte response is rapidly recruited to the CNS that enhances neurological disease by immune mediated CNS tissue destruction. In the absence of functional B lymphocytes this process will lead to a very rapid clinical deterioration and death of the animals without clearance of virus from the tissue. In presence of a functional B cell compartment, however, extracellular spread of the virus can be stopped if antibody secreting cells penetrate into the CNS at an opportune time span past infection. As a consequence T lymphocyte-mediated tissue damage is restricted to an extent that finally allows clearance of virus from the brain and recovery from disease.

Results

To examine the impact of lymphocyte subsets on the onset and the early clinical course of intracerebral infection with coronavirus JHM, we monitored the development of neurological symptoms and the viral load in brain tissue of irradiated animals which were either completely reconstituted by transfer of leukocytes, left unreconstituted or were partially reconstituted before infection by naive lymphocytes.

Immunocompetent animals

As seen routinely in our laboratory, JHMV-infected, non-irradiated rats typically developed mild signs of paresis at day 8 p.i. with an increase in severity of symptoms the next day (Figure 1a). Complete reconstitution of the animals by transfer of unseparated spleen and lymph node cells resulted in a comparable clinical course to that of non-irradiated animals (Figure 1a). As depicted in Figure 2, attempts to isolate infectious virus from brain tissue of both cohorts of animals at 9 d.p.i. regularly failed (detection level $0.5 \cdot 10^3$ $TCID_{50}$/g of CNS).

Figure 3a shows typical tissue damage in the CNS of a non-irradiated JHMV-infected LEW rat 9 d.p.i. Small, but distinct vacuoles were scattered through the white matter of the cerebellum. Irradiation and complete reconstitution of animals with lymph node and spleen cells, resulted in a comparable picture (Figure 3b).

Immunodeficient animals

Surprisingly, animals that were left completely unreconstituted after irradiation and infected by JHMV developed signs of mild neurological disease as late as fully competent rats (Figure 1b). However, it was of note that a different clinical picture emerged in immunodeficient animals. Instead of paralytic symptoms, an increasing degree of hyperexcitation was noticed, characterized by motoric restlessness and tremor. Up to 9 d.p.i. these changes were mild, but in animals which occasionally were observed up to 14 days neurological disease gradually increased in severity (data not shown). As could be expected in the absence of a functional immune system, the yield of recoverable virus from brain tissue was high at 9 d.p.i. As much as $9.2 \cdot 10^3$ $TCID_{50}$/g of CNS was detectable (Figure 2). Despite this strong virus replication, tissue destruction was rather unspectacular. Only occasionally swollen

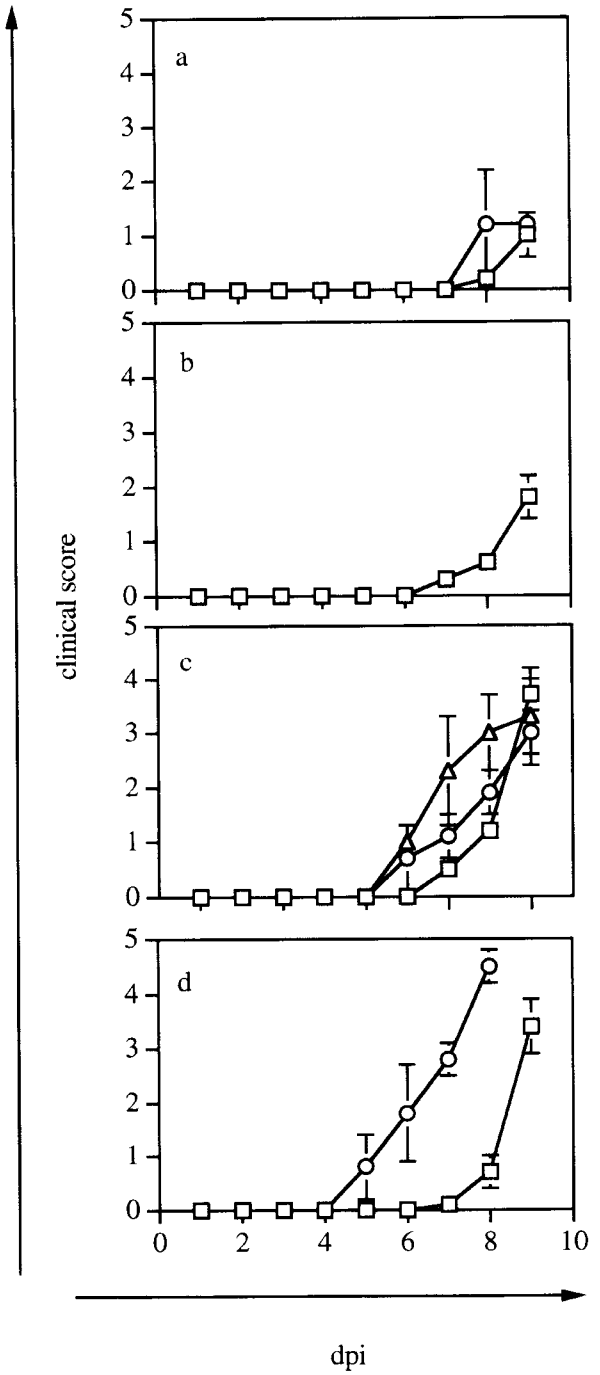

Figure 1 Clinical course of intracerebral JHMV infection in LEW rats. (**a**) Twenty-four hours prior to infection with JHMV, animals were untreated (□) or γ-irradiated with 6 Gy and immediately after irradiation intravenously reconstituted with spleen and lymph node cells (○); (b) Twenty-four hours before infection with JHMV animals were γ-irradiated with 6 Gy (**c**) Twenty-four hours before infection with JHMV animals were γ-irradiated with 6 Gy and immediately after irradiation intravenously reconstituted with naïve $CD4^+$ and $CD8^+$ T cells (□) or with individual T cell subsets alone ($CD4^+$ cells ○, $CD8^+$ cells △); (**d**) same treatment as under (**b**), but different lymphocyte subsets transferred before infection (B and $CD8^+$ cells ○, B and $CD4^+$ cells □). For amounts of transferred cells see Material and methods section. Clinical scoring was performed daily and plotted *versus* time past infection. Clinical score for **a** and **c** to h: (0) no clinical signs; (1) uncommon social behavior, loss of tail tone; (2) paresis of one or two legs; (3) paresis of more than two legs or paralysis of one or two legs; (4) tetraplegia; (5) moribund state. Clinical score for **b**: (0) no clinical signs; (1) uncommon social behavior; (2) restlessness, enhanced sensitivity to touch and noise; (3) tremor; (4) hyperexcitated running and jumping. SE was determined from 5 to 14 identically treated animals out of two experiments.

axons, oedema and subtle vacuolization of myelin sheaths were seen (Figure 3c). Thus, tissue alterations were almost undistinguishable from the mild changes due to irradiation without infection (Figure 3d).

T cell subset reconstituted animals

Extremely severe paralytic symptoms prevailed in animals that received either $CD4^+$ T lymphocyte enriched cells, $CD8^+$ T lymphocyte enriched cells or both together (Figure 1c). Predominantly in animals which were reconstituted either by $CD4^+$ or $CD8^+$ T lymphocytes, neurological signs started already at 6 d.p.i. with a fast progression to a moribund state. In animals where both T lymphocyte subsets were transferred simultaneously a

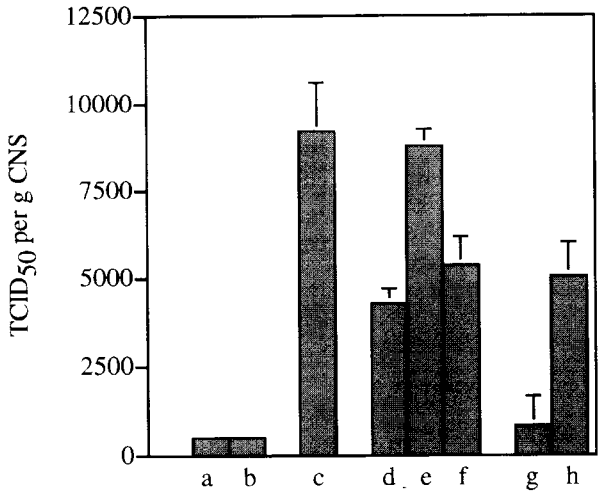

Figure 2 Virus recovery from the central nervous system of JMV infected LEW rats. Before infection with JHMV, animals were: (a) left untreated; (b–h) γ-irradiated with 6 Gy. Immediately after irradiation animals were (**c**) not reconstituted; reconstituted with (b) spleen and lymph node cells; (d) $CD4^+$ T cells; (e) $CD8^+$ T cells; (f) $CD4^+$ and $CD8^+$ T cells; (g) $CD4^+$ T cells and sIg^+ B cells; (h) $CD8^+$ T cells and sIg^+ B cells. Inoculation of animals with JHMV was done 24 h past reconstitution by lymphocytes. For amount of transferred lymphocytes see Material and methods. Animals were sacrificed 9 d.p.i. and $TCID_{50}$ of JHMV were titrated from the entire CNS. SE is calculated from at least three identically treated animals observed in two independent experiments.

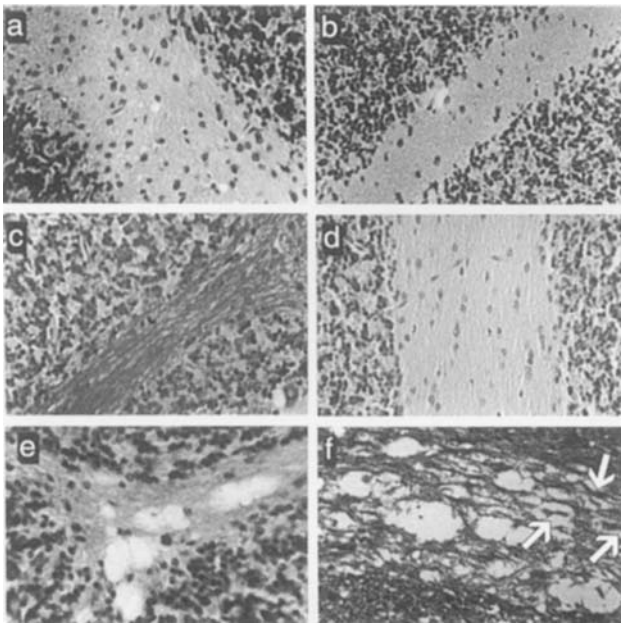

Figure 3 Histopathological changes in the cerebellum of JHMV-infected LEW rats at 9 d.p.i. (**a**) JHMV infection alone; (**b**) irradiation, complete reconstitution by transfer of spleen and lymph node cells followed by infection; (**c**) irradiation, no reconstitution and infection; (**d**) or irradiation alone introduced no histopathological alterations to the inner layer of the cerebellum at 9 days past infection (all pictures: LFB staining, magnification 250-fold); (**e**) irradiation, $CD4^+$ reconstitution and infection resulted in severe vacuolization of the inner molecular layer of the cerebellum at 9 days past infection (LFB staining, magnification 400-fold). (**f**) Myelin sheaths in the inner molecular layer of an animal irradiated, reconstituted by $CD8^+$ T cells and infected by JHMV were blown up often containing axon fragments as indicated by arrows (LFB staining, magnification 400-fold).

clinical score of almost 4 at 9 d.p.i. exemplified again strong neuropathogenic properties of T lymphocytes.

To exclude, that these symptoms can be induced also by irradiation and transfer of lymphocyte subsets without subsequent infection, control animals were examined which were irradiated and reconstituted by either $CD4^+$ or $CD8^+$ T lymphocytes but not infected with JHMV. Up to 9 d.p.i. a slight anemia and very subtle signs of irradiation damage (enhanced sensitivity to touch and noise) but no paralytic signs of disease were observed in these animals (data not shown).

Compared to immunodeficient animals only slightly less virus was recovered in irradiated rats, reconstituted with $CD8^+$ T cells ($8.8 \cdot 10^3$ $TCID_{50}$/g CNS). In contrast, a moderate reduction of viral load was observed in rats receiving only $CD4^+$ T lymphocytes or a combination of $CD4^+$ and $CD8^+$ T lymphocyte enriched cells ($4.3 \cdot 10^3$ $TCID_{50}$/g CNS and $5.4 \cdot 10^3$ $TCID_{50}$/g CNS, respectively).

As expected, the severe clinical course of the infection was reflected in strong CNS tissue destruction. Nevertheless, subtle differences were noticed between animals which were $CD4^+$ T cell reconstituted and animals which received $CD8^+$ T cells. Characteristically, $CD4^+$ T cell reconstituted animals disclosed huge vacuoles in distinct clusters in the white matter of (Figure 3e). In contrast, in the presence of $CD8^+$ T lymphocytes vacuolization of the white matter was widespread throughout brain stem and spinal cord accompanied by some perivascular cuffs (Figure 3f).

T cell subset reconstituted animals co-transferred with B cells

The effects of co-transfer of T and B lymphocytes on the clinical course of the infection was dependent on the accompanying T lymphocyte subset (Figure 1d). The combined transfer of $CD4^+$ T lymphocytes and B lymphocytes resulted in a remarkable shift of disease onset to 7 d.p.i. (Figure 1d), which is the same time past infection when immunocompetent animals reveal first overt clinical signs of the infection (Figure 1a). As expected viral load was low in the brain of $CD4^+$ T/B cell reconstituted animals but interestingly this could not prevent further development of neurological disease and sometimes a lethal outcome of infection at 9 d.p.i. (Figure 1c).

Compared to animals reconstituted by $CD8^+$ T cells only there was no beneficial effect on the disease process by co-transfer of $OX33^+$ B lymphocytes. On the contrary, it seemed that onset of neurological signs was earlier (5 d.p.i.) and progression of the disease to a disastrous clinical state (average score of 4) was even faster compared to $CD8^+$ T lymphocyte substituted animals (Figure 1c). Surprisingly, transfer of $CD8^+$ T lymphocyte enriched cells together with B lymphocytes resulted in a moderate but significant reduction of viral load compared to animals which received only $CD8^+$ T lymphocyte enriched cells.

Significance of differences in the development of neurological disease

For statistical evaluation, the independent Student's *t*-test was applied. No significant differences were detected 9 d.p.i. between untreated, JHMV infected animals and those that were irradiated, reconstituted by spleen and lymph node cells and infected ($P > 0.05$). However, highly significant differences could be verified between untreated, JHMV infected animals and animals that were irradiated and reconstituted before infection by transfer of $CD4^+$ and $CD8^+$ T cells ($P < 0.005$), of $CD4^+$ T cells ($P < 0.005$), of $CD8^+$ T cells ($P < 0.005$), of $CD4^+$ T cells and B cells ($P < 0.005$) and of $CD8^+$ T cells and B cells ($P < 0.005$). The same holds true for irradiated animals reconstituted before infection by transfer of $CD4^+$ and $CD8^+$ T cells and those

that received spleen and lymph node cells ($P<0.005$).

Discussion

Since the reappraisal of coronavirus JHM induced encephalomyelitis in mice as an animal model for virus-induced primary demyelination (Weiner, 1973) it was generally assumed that its pathogenesis is primarily determined by cytopathogenic properties of the virus for oligodendrocytes. Only recently, immune effector mechanisms are considered to be of importance for the outcome of intracerebral JHMV infection in rodents (Dörries, 1990; Houtman and Fleming, 1996b). In line with these arguments are our data collected from animals that were irradiated but not reconstituted by lymphocyte transfer before JHMV infection. Despite a high load of infectious virus in the CNS of these animals, onset as well as progression of neurological disease was slow. Moreover, the quality of neurological disease was quite different from that of immunocompetent rats. Instead of a paralytic disease, which usually indicates white matter destruction, a mild encephalitic course was observed, pointing to affection of neurons, rather than to primary demyelination. This finding in combination with data of others (Fleming *et al*, 1993; Houtman and Fleming, 1996a; Wang *et al*, 1990) who demonstrated the absence of myelin damage and high viral load in JHMV infected mice that were severely immunocompromised, support the speculation that immunodeficient Lewis rats probably will not develop primary demyelination later on in the course of infection. However, as pointed out by Houtman and Fleming (1996b) the pathogenesis of JHMV-induced encephalomyelitis can be dissected into three subsequent disease patterns: acute encephalomyelitis, viral clearance and demyelination. All these processes are linked more or less to the immune system response, but the event of demyelination must not necessarily be immune-mediated. As a matter of fact it can be completely independent from any immune system action as was shown unequivocally by others (Houtman and Fleming, 1996a; Sorensen *et al*, 1987; Sutherland *et al*, 1997). Nevertheless, these findings do not exclude that early T cell-mediated clearance of the virus may contribute to the initiation of demyelinating disease. The latter is supported by our data presented here, which suggest white matter tissue destructive and disease enhancing properties of early infiltrating $CD4^+$ and/or $CD8^+$ lymphocytes.

In contrast to immunodeficient LEW rats, a significantly reduced titer of CNS recoverable virus in irradiated rats that were reconstituted only by $CD4^+$ cells was accompanied by enhancement of acute neurological disease. So far, the nature of the virostatic effector mechanisms exerted by $CD4^+$ T cells remains unclear but work is in progress to examine the pattern of cytokine-specific mRNA expression in CNS-infiltrating T cells by reverse transcription and polymerase chain reaction. Since we have observed earlier that upregulation of MHC class II antigens in the course of JHMV infection is particularly notable on CNS-resident microglia cells (Sedgwick *et al*, 1991), it is tentative to speculate that antiviral action of $CD4^+$ T lymphocytes might be mediated indirectly via activation of microglia cells. In this case interferon-γ would be a prime candidate for the effective cytokine, and preliminary unpublished data from our laboratory disclose indeed that interferon-γ specific mRNA is strongly expressed in CNS infiltrating T cells. This hypothesis is supported by work from Pearce *et al* (1995), who showed upregulation of interferon-γ mRNA in the CNS of immunocompetent mice after infection with MHV and by the fact that interferon-γ is important for efficient viral clearance of MHV infection in mice (Smith *et al*, 1991). Moreover, as shown by Kündig *et al* (1993), interferon-γ exerts antiviral activity in brain tissue.

$CD8^+$ T cells alone, which were highly disease-inducing, could not restrict viral replication efficiently. The same phenomenon was observed in JHMV infected mice by Williamson and Stohlman (1990) and recent data from the same laboratory suggest that in $CD4^+$ T cell depleted animals $CD8^+$ T cells can enter the CNS but due to rapid apoptosis they can not exert their effector functions in brain tissue (Stohlman *et al*, 1998). Nevertheless, in view of other model systems (Buller *et al*, 1987; Matloubian *et al*, 1994; Rahemtulla *et al*, 1991) and our data presented here we tend to believe that $CD8^+$ T cells can differentiate into effector cells without assistance of $CD4^+$ T cells, because the most severe clinical courses were observed in animals that were supplied by $CD8^+$ T cells only. The entangling finding of pathogenic $CD8^+$ T cell action without viral clearance in the absence of $CD4^+$ T cells might be explained by a lack of interferon-γ secreting virostatic $CD4^+$ T cells. This would also fit in our observation that MHC antigens are only moderately upregulated in $CD8^+$ T cell reconstituted animals (data not shown).

Apparently contradictory to our findings about the role of T cells in JHMV-induced encephalomyelitis are reports from different laboratories, that transfer of $CD4^+$ as well as $CD8^+$ T lymphocytes is of therapeutic value for JHMV-infected rats and mice, implicating a 'protective' function of T cells (Erlich *et al*, 1989; Körner *et al*, 1991; Stohlman *et al*, 1995; Sussman *et al*, 1989; Yamaguchi *et al*, 1991). This protective character of T lymphocytes seems to be further confirmed by depletion studies in mice, where it was shown that JHMV-infection after *in vivo* depletion of either $CD4^+$ or $CD8^+$ T cells before infection resulted in the abolishment of viral clearance from the CNS in mice (Williamson and

Stohlman, 1990) and that recombinant vaccinia/JHM virus vaccinated rats succumb to an intracerebral challenge with JHMV when depleted from $CD8^+$ cells (Flory *et al.*, 1993).

Evaluation and comparison of the accumulated data, however, reveal that T lymphocytes are neither protective nor pathogenic *per se*. Circumstances like the state of T cell differentiation and degree of viral dissemination in the CNS will determine if T cell mediated effector functions in JHMV infected brain tissue will protect from acute disease or will enhance neurological symptomatology.

In all cases where T cells were primed by viral antigens before transfer and were given to the animals before, concomitantly or shortly after intracerebral JHMV infection (Erlich *et al*, 1989; Körner *et al*, 1991; Sussman *et al*, 1989; Yamaguchi *et al*, 1991), the primed state of the transferred T cell population enables immediate antiviral action either by $CD8^+$ T cell mediated viral clearance or $CD4^+$ T lymphocyte assisted rapid recruitment of the virus-specific antibody response as well as release of virostatic cytokines. In any case, it is the prompt and effective restriction of viral spread in the tissue that keeps action of T effector cells on a subclinical level and thus explains the protective nature of T lymphocytes. This interpretation is further supported by the observation that transfer of JHMV-specific T cells 2 d.p.i. or later does not protect mice from a lethal outcome of the infection (Yamaguchi *et al*, 1991).

In contrast, as shown in this paper, JHMV infection of LEW rats with a naive T cell compartment, that is either incomplete or not supplemented by B lymphocytes results in different effects on onset and course of disease. Compared to animals after adoptive transfer of virus-specific T cells or T cell lines, in naive animals peripheral priming, clonal expansion and differentiation of T lymphocytes almost certainly causes a considerable delay in the recruitment of immune effector cells to the brain parenchyma. This allows initial spread of the virus undisturbed by adaptive immune effector mechanisms. Subsequently, action of primed and activated T effector cells causes strong tissue destruction and thereby neurological disease. As shown here, in the absence of B lymphocytes this process will eventually cause death of the animal, because extracellular distribution of virus cannot be controlled completely by T cells alone.

The important role of B lymphocytes for dampening of disease and final recovery from infection is strongly supported by our data and is in agreement with previous findings generated in our laboratory. Complete reconstitution of animals by spleen and lymph node cells before infection, resulted in an absolutely comparable course of the infection as seen in unmanipulated animals. Moreover, we observed earlier that convalescence of JHMV infected LEW rats is always associated with the appearance of virus-specific antibody secreting cells in the CNS and that in Brown Norway (BN) rats, which are clinically resistant to JHMV-infection, the rapid and effective recruitment of virus-specific antibody secreting cells to the CNS results in high titers of JHMV-specific antibodies in cerebrospinal fluid (Dörries *et al*, 1987; Schwender *et al*, 1991). However, as illustrated by the animals that were reconstituted only by $CD4^+$ T cells and B lymphocytes, onset of neurological disease can be delayed significantly in the presence of B cells, but this is not sufficient for complete recovery from disease. This is in line with findings of Williamson and Stohlman (1990), who showed in mice that complete clearance of the virus from the CNS is only achieved when cytotoxic $CD8^+$ T lymphocytes are available. Most likely, it is the lack of complete eradication of virus-producing cells in the tissue which allows progression of disease. Thus, it needs action of all lymphocyte subsets in a coordinated fashion to recover from JHMV-induced encephalomyelitis.

At present, we have no conclusive explanation for the observation that animals which were reconstituted by $CD8^+$ T cells and B cells show very severe clinical signs of infection but moderate reduction of viral load in the brain compared to animals which received $CD8^+$ T lymphocyte enriched cells alone. Recently, it was shown by Maloy *et al* (1998) that mice can synthesize virus-specific antibodies in the absence of a functional α/β^+ T cell compartment by help of γ/δ^+ T cells. Thus, although highly speculative, it could be hypothesized that γ/δ^+ T cells interact with B lymphocytes in the co-transferred animals. However, no data can be provided to support this assumption so far.

In conclusion, all data accumulated in the rodent/MHV model systems, suggest that the impact of the immune system response on the course of JHMV-induced encephalomyelitis most likely was underestimated in the past. If unbalanced, intracerebral T cell immune effector mechanisms can be lethal. On the other hand, fine tuning of both, cellular and humoral immune effector functions is indispensable for LEW rats to survive intracerebral JHMV infection.

Material and methods

Virus and animals

Animal experiments were approved by the appropriate authorities and conducted in accordance with legal rules. The animals were obtained as specifically pathogen free from 'Deutsches Zentralinstitut für Versuchstierzucht' (Hannover, Germany) and kept under sterile conditions.

Coronavirus JHM was propagated and purified as described previously (Schwender *et al*, 1991). Briefly, the virus was passaged once through

embryonic mouse brain followed by one passage through sac (−) cells. Cell free supernatant was taken from this tissue culture and diluted with MEM to contain 10^4 $TCID_{50}$ of JHM virus/ml. For infection, 100 μl thereof were injected into the left brain hemisphere of LEW rats at the age of 3 weeks. Inoculation of animals with virus was performed on the next day following γ-irradiation and reconstitution by lymphocyte transfer.

Irradiation of animals

Animals were whole body γ-irradiated with a sublethal dose of 6 Gy. Effects of the irradiation procedure on immunocompetence were checked in spleenocytes and lymph node cells by measuring ^{3}H-thymidine incorporation after Con A stimulation (Imrich *et al*, 1994) and determination of antigen-specific antibody secreting plasma cells by ELISpot assay (Schwender *et al*, 1991) after a single intraperitoneal immunization with 200 μg of key-hole limpet hemocyanine (KLH) in 1.0 ml of PBS. Additionally, KLH-specific antibodies were titered in serum specimens by EIA standard procedures. For determination of the proliferative Con A response, lymphocytes ($1.0 \cdot 10^6$/ml) were incubated in duplicates for 48 h in RPMI medium (100 μl/ml) supplemented with Con A (2.0 μg/ml) and 10% FCS. Tritiated thymidine (14.8 kBq/well, specific activity 1.59 TBq/mMol) was added for the last 24 h of culture. DNA incorporated ^{3}H-thymidine was determined by harvest and hypotonic lysis of cells on glass fiber filters followed by measurement of filter bound radioactivity.

After irradiation, ^{3}H-thymidine incorporation in lymphocytes was reduced to 0.2% of the value detectable in cells from untreated animals (data not shown). Moreover, compared to untreated animals, only 0.02% of KLH-specific IgM-secreting plasma cells and no KLH-specific IgG antibody secretors were detected in irradiated rats.

Reconstitution of animals

Immediately after irradiation, lymphocyte subpopulations were transferred through the lateral tail vein into ether anesthetized animals. Transferred lymphocytes were isolated by standard protocols from spleen, cervical lymph nodes (LN) and mesenterial LN of uninfected syngeneic animals of matching age. These cells were either non separated lymphocytes, $CD4^+$ T lymphocyte or $CD8^+$ T lymphocyte enriched cells, or $CD4^+$ T lymphocyte and/or $CD8^+$ T lymphocyte enriched cells in combination with B lymphocyte enriched cells. Preparation of cell populations was performed by negative selection using immunomagnetic procedures following the manufacturers' instructions (DRG, Wiesbaden, Germany and Dianova, Hamburg, Germany). Mouse monoclonal antibodies (mabs) used for primary labeling of lymphocyte subpopulations were CD4-specific W3/25 (Williams *et al*, 1977), CD8 specific OX8 (Brideau *et al*, 1980) and B lymphocyte LCA-specific OX33 (Woollett *et al*, 1985). Depletion of mab tagged lymphocyte subsets was achieved by two cycles of incubation with magnetic beads coupled with rabbit anti-mouse IgG and subsequent exposure of the cell suspension to a magnetic field. Unlabeled cells were removed, quantitated by trypan blue exclusion and checked for purity by flow cytometry after labeling with the appropriate FITC-labeled antibody. Especially, care was taken to avoid contamination of $CD8^+$ T lymphocytes by $CD8^+$ natural killer (NK) cells. Since $CD8^+$ NK cells are $CD8^{low}$ compared to $CD8^{high}$ T cells (Chambers *et al*, 1989) contamination of $CD8^+$ T lymphocytes by $CD8^+$ NK cells was efficiently controlled by a single staining procedure for CD8 followed by flow cytometric analysis. After preparation of $CD8^+$ cell populations, we never have observed a significant contamination by $CD8^{low}$ NK cells (data not shown). This phenomenon is probably due to the preparation protocol. Cells enriched for $CD8^+$ T lymphocytes were prepared by depleting $CD4^+$ T cells and B lymphocytes using mouse monoclonal antibodies and anti-mouse IgG antibodies. Usually, unspecific labeling of cells due to cross reactivity of anti-mouse IgG with rat IgG is blocked by addition of rat IgG to the incubation buffer. However, since we did not add rat IgG, treatment by anti-mouse IgG helped to remove Fc-receptor$^+$ NK cells which carry mouse IgG in their Fc-receptors. Only cell preparations containing less than 1% contamination with respect to the population to be depleted were used for transfer (i.e. typically, an $CD4^+$ T lymphocyte enriched cell preparation consisted of 90–95% $CD4^+$ cells, $<1\%$ $CD8^+$ cells, $<1\%$ sIg^+ cells and approximately 3–8% of untyped cells). Each irradiated animal received an amount of enriched cells, that corresponded to their proportion in $2.0 \cdot 10^8$ unseparated, naive lymphocytes (i.e. $1.0 \cdot 10^8$ $CD4^+$ cells if 50% of the unseparated cell preparation were $CD4^+$).

Scoring of neurological disease

Animals that were monitored for clinical signs of neurological disorders every day after infection using the following previously published scoring system (Dörries *et al*, 1991): (0) no clinical signs; (1) uncommon social behavior, loss of tail tone; (2) paresis of one or two legs; (3) paresis of more than two legs or paralysis of one or two legs; (4) tetraplegia; (5) moribund state. In irradiated animals which were not reconstituted by lymphocytes but infected by JHMV a different type of disease prevailed. Thus, the scoring system was adapted to this situation: (0) no clinical signs; (1) uncommon social behavior; (2) restlessness, enhanced sensitivity to touch and noise; (3) tremor; (4) hyperexcitated running and jumping. The average of the clinical scores was determined for each day past infection.

Titration of infectious virus
Infectious virus was titered in brain and spinal cord from animals sacrificed at day 9 past infection. For that purpose, the weight of each CNS was determined and after adding the same amount of MEM (5% FCS) (w/v) the tissue was homogenized in a 5 ml Dounce homogenizer. The suspension was clarified by centrifugation (30 min, 12 500· g), the supernatant collected and serially diluted. Aliquots of diluted supernatant were assayed in triplicates for presence of infectious virus on a microtiter plate using DBT-cells as target cells (Hirano *et al*, 1974). The $TCID_{50}$ was regarded as the highest dilution where DBT-monolayers in at least two of three wells disclosed more than 50% cytopathic effect.

Histology
Animals were perfused with 200 ml of formaldehyde (2%) and brains were examined by serial sections. Histopathological alterations were assessed with the help of luxol fast blue (LFB) and hematoxilin/eosin stainings according to standard protocols.

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