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IgG subclass responses to Theiler's murine encephalomyelitis virus infection and immunization suggest a dominant role for Th1 cells in susceptible mouse strains

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SUMMARY

Inbred mouse strains differ in susceptibility to Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease. A strong correlation between disease susceptibility and delayed-type hypersensitivity (DTH) has been previously demonstrated, but no strong correlation between disease susceptibility and total anti-TMEV ELISA titres was shown. Since both DTH and IgG2a antibody production are regulated by $CD4^+$ Th1 cells, we investigated three strains of mice to determine whether antivirus IgG2a antibody levels, like DTH in previous studies, correlated with disease susceptibility. Susceptible SJL/J, intermediately susceptible C3H/HeJ, and resistant C57BL/6 mice were infected intracerebrally (i.c.) with the BeAn strain of TMEV and monitored for clinical signs of demyelination and for levels of TMEV-specific antibody of different IgG subclasses using a particle concentration fluorescence immunoassay (PCFIA). Resistant C57BL/6 mice were found to have significantly lower concentrations of total anti-TMEV antibody than susceptible SJL/J mice and intermediately susceptible C3H/HeJ mice show variable antibody responses. A predominance of anti-TMEV IgG2a (Th1 regulated) antibody was seen in susceptible and intermediately susceptible mice, whereas resistant mice displayed a predominant anti-TMEV IgG1 (Th2 regulated) response accompanied by a marked deficiency of IgG2a. In contrast, immunization of C57BL/6 mice with UV-inactivated TMEV in adjuvant revealed that this strain was not defective either in its ability to generate high levels of anti-TMEV antibody or in its ability to produce IgG2a antibody. These results suggest that the antivirus IgG subclass profile is dependent upon the immunization route, virus viability and/or the use of adjuvant and that the levels of antivirus subclasses may be predictive of disease susceptibility.

INTRODUCTION

Experimental infection of susceptible inbred strains of mice with Theiler's murine encephalomyelitis virus (TMEV) leads to a chronic, immune-mediated, inflammatory demyelinating disease within 1–2 months.[1-4] Following infection, low levels of virus persist in the central nervous system (CNS) of both disease-susceptible and resistant mice for virtually their entire lifetime.[2,3] Virus replication is limited to the spinal cord white matter, and focal inflammation associated with collections of mononuclear cells, macrophages, and reactive astrocytes can be found in the leptomeninges and white matter. From 3 to 5 months post-infection, extensive demyelination occurs, with lipid-laden macrophages present in lesions, with lesions virtually replacing the white matter with little effect on neurons and blood vessels.[1,2] Myelin breakdown clearly leads to clinical symptoms including gait spasticity, urinary incontinence and spastic paralysis.

The characteristic infiltration of mononuclear cells into the CNS during the demyelinating phase of TMEV-induced disease suggested a model[5] wherein virus-specific delayed-type hypersensitivity (DTH) within the CNS results in demyelination by a terminal, non-specific, macrophage-mediated, bystander response. This is supported by findings that disease susceptibility correlates with the temporal development of chronic, high levels of TMEV-specific, major histocompatibility complex (MHC) class II-restricted DTH responses, but not with splenic T-cell proliferative responses or CNS virus titres.[5-7]

TMEV-induced demyelinating disease is an excellent experimental animal model for human multiple sclerosis (MS) because

Abbreviations: ADDC, antibody-dependent cell-mediated cytotoxicity; CNS, central nervous system; DTH, delayed-type hypersensitivity; i.c., intracerebral; MS, multiple sclerosis; PCFIA, particle concentration fluorescence immunoassay; TMEV, Theiler's murine encephalomyelitis virus.

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of histopathological and genetic similarities, and by epidemiological studies favouring a virus aetiology of MS.[8,9] The role of antibodies in these two diseases remains unclear. We previously demonstrated that ELISA antibody titres in genetically disparate inbred mouse strains weakly correlated with susceptibility to TMEV-induced disease, however both resistant and susceptible inbred strains produced very high titres of antibody 3–4 months post-infection as measured by this assay.[7] However, no correlation of susceptibility to anti-TMEV titres was demonstrable in susceptible and resistant strains with limited genetic differences.[5] However, it has been reported that partial anti-μ suppression of antivirus antibody responses in TMEV-infected susceptible SJL/J mice aggravated clinical and histological disease.[10] This raises some important questions regarding the potential role for antibody in either pathogenesis and/or protection from disease.

Murine CD4+ T cells of the Th2 subset regulate IgG1 and IgE production by interleukin-4 (IL-4), whereas CD4+ Th1 cells appear to mediate DTH and regulate IgG2a production via interferon-gamma (IFN-γ) production.[11–13] Immune responses to most antigens are balanced between cell-mediated immunity (CMI) and antibody production. However, in some situations, including infections with certain bacterial, protozoan and virus agents, preferential stimulation of Th1-like or Th2-like patterns of cytokine synthesis *in vivo* leads to immune responses dominated by either CMI or antibody production.[14–19] Since TMEV-susceptible mouse strains show apparent preferential activation of the Th1-like cells following virus infection as evidenced by elevated *in vivo* virus-specific DTH[5–7] and *in vitro* IFN-γ production (S. D. Miller, unpublished results), there could be preferential *in vivo* production of IgG2a antibody responses. In this study we examined the virus-specific IgG subclass responses of susceptible SJL/J, intermediately susceptible C3H/HeJ, and resistant C57BL/6 mice following both infection and immunization with TMEV using a quantitative particle concentration fluorescence immunoassay (PCFIA). The data show that following infection of both SJL/J and C3H/HeJ mice an IgG2a antivirus response predominates, whereas in resistant C57BL/6 mice an IgG1 response predominates. However, C57BL/6 mice have the genetic capacity to make high levels of antivirus IgG2a antibody following peripheral immunization with UV-inactivated TMEV in complete Freund's adjuvant (CFA) indicating that factors other than host genetics can influence cytokine synthesis patterns and hence the type of immune response observed.

MATERIALS AND METHODS

Mice

Female SJL/J, C57BL/6, and C3H/HeJ mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice used were 4–6 weeks old at initiation of these experiments and were maintained on standard laboratory chow and water *ad libitum*.

Inoculation, immunization and examination of mice

Mice were anaesthetized with Methoxyflurane and inoculated in the right cerebral hemisphere with $1{\cdot}3 \times 10^6$ plaque-forming units (PFU) of the BeAn stain of TMEV in 30 μl. Control mice received 30 μl of phosphate-buffered saline (PBS). Mice were examined several times per week for the first 3 weeks and once weekly thereafter for development of neurological signs, particularly the chronic gait abnormality and spastic paralysis which are indicative of demyelination. Mice were immunized subcutaneously (s.c.) with 25 μg of TMEV in CFA on Day 0, followed by intraperitoneal immunization with 25 μg of TMEV in Maalox (aluminum–magnesium hydroxide gel; Wm F. Rover, Fort Washington, PA) on Days 14 and 21.

Serum collection

Blood was collected from the retro-orbital plexus of anaesthetized mice, serum prepared, and stored at $-20°$. Blood was drawn from each experimental and control mouse at regular time intervals.

Virus preparation

Virus was prepared as described previously.[4,20] Briefly, confluent BHK-21 cells were infected with TMEV, BeAn 8386 strain, in tissue culture medium for 48 hr. Virus in the supernatant and cellular debris was precipitated with NaCl and polyethylene glycol (PEG). The PEG precipitate, containing virus and cellular debris, was pelleted by centrifugation and resuspended by sonication. Virus was further purified by ultracentrifugation on sequential discontinuous 15–30% sucrose and Cs_2SO_4 gradients. Finally, the virus was pelleted, resuspended and measured for optical absorbance at $A_{(280)}$ to determine concentration. Ultraviolet (UV) inactivation of virus was performed according to established methods which successfully kill virus as measured by a plaque assay on baby hamster kidney cells.

Assay of TMEV-specific serum antibody concentrations

Serum antibody levels of individual experimental and control mice to the BeAn strain of TMEV were quantitated using a modified, fluid-phase PCFIA as previously described.[20,21] PCFIA is more rapid, more sensitive, and has significantly lower background signals than similarly performed ELISA.[20] Briefly, goat anti-mouse immunoglobulin (Ig) subclass particles were prepared by incubating avidin-conjugated polystyrene particles (0·25% w/v) with a 1/200 dilution of biotin-labelled, affinity purified, goat anti-mouse IgG subclass antibodies (b-anti-IgG1, b-anti-IgG2a, or b-anti-IgG2b purchased from Caltag Laboratories (South San Francisco, CA) at room temperature with occasional mixing. Serial dilutions ($\log_2$) of mouse sera (20 μl/well) were incubated with 20 μl PBS containing 40 ng of FITC-labelled TMEV in 96-well Fluoricon Assay Plates (Pandex, Mundelein, IL) for 30 min at room temperature. Twenty microlitres of goat anti-mouse Ig subclass particles [0·25% (w/v) suspension] was added to each well and the plates incubated for an additional 30 min at room temperature. A Fluorescence Concentration Analyzer (Pandex) was used for phase separation, washing, FITC excitation (485 nm) and determination of relative fluorescence units (RFU) light emission (535 nm). Antibody concentrations of individual serum samples were determined by logarithmic linear regression analysis of the RFU values using a computer program developed in our laboratory. In all cases the correlation coefficient (r) for the linear portions (3–5 points) of the titration curves were $-0{\cdot}95$ or better. The results are expressed as the mean concentration of total anti-TMEV antibody in μg/ml or as the IgG subclass percentages of total antivirus antibody $\pm$ SEM based upon a standard curve obtained by PCFIA analysis of a hyperimmune serum sample.

Statistical analyses

Comparisons of the antibody levels between different mouse strains were analysed using the Student's *t*-test. Two-tailed *P* values of $<0{\cdot}05$ were considered significant.

RESULTS

Total anti-TMEV antibody and clinical course of disease in SJL/J, C3H/HeJ and C57BL/6 mice

SJL/J, C3H/HeJ and C57BL/6 mice were infected i.c. with TMEV and observed for 140 days for development of clinical demyelinating disease. Control mice were i.c. injected with PBS and showed no clinical disease (data not shown). As shown in the left panels of Fig. 1a, b, in two experiments SJL/J mice were highly susceptible to the induction of demyelinating disease with 66 and 90% of the mice displaying clinical signs with a mean day of onset of 30 days post-infection. C3H/HeJ mice displayed intermediate susceptibility in that approximately 20% (Fig. 1a) and 33% (Fig. 1b) of the mice developed disease with a mean day of onset between 35 and 70 days post-infection. In contrast the C57BL/6 mice were totally resistant as they showed no clinical signs of demyelination.

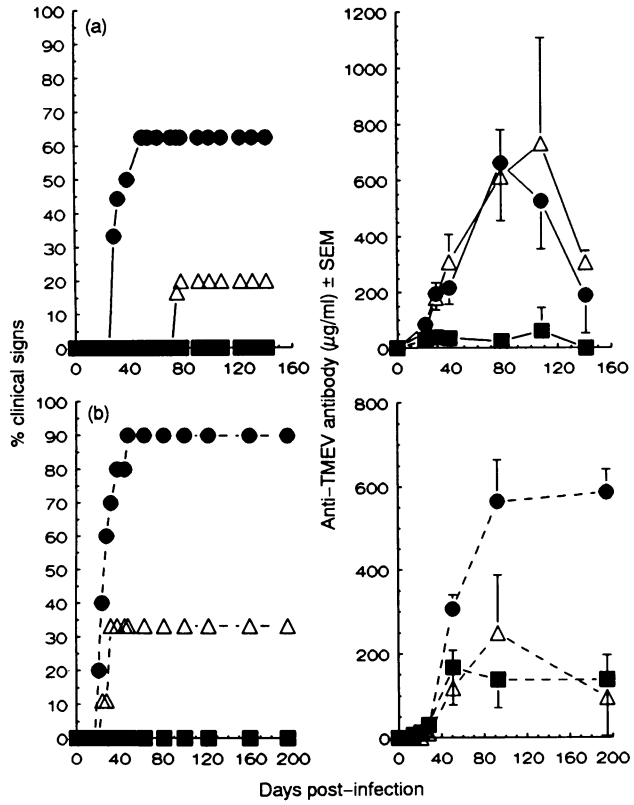

Figure 1. Total anti-TMEV antibody levels and clinical course of disease in TMEV-infected SJL/J (●), C3H/HeJ (△) and C57BL/6 (■) mice. Groups of nine to 10 SJL/J, C3H/HeJ and C57BL/6 mice were infected i.c. with $1{\cdot}3 \times 10^6$ PFU of the BeAn strain of TMEV. Control mice were i.c. injected with PBS and showed no clinical disease and no TMEV-specific antibody (data not shown). Data from two separate experiments are shown. The left panel of each experiment indicates the percentage of TMEV-infected mice displaying clinical signs of demyelination (i.e. waddling gait). The right panel shows the mean total anti-TMEV antibody responses of individual mice measured by PCFIA at 21, 29, 39, 78, 108 and 141 days post-infection (a—Exp. 1) and at 14, 21, 28, 51, 94 and 196 days post-infection (b—Exp 2). Antibody data are expressed as μg/ml ± SEM.

Quantitative analysis of the total antibody responses of these mice by a modified PCFIA[20,21] at different time-points during the clinical course of disease confirms our earlier observation[7] that susceptible SJL/J mice show significantly higher antibody levels than seen in resistant C57BL/6 mice (Fig. 1a, b, right panels). However, PCFIA analyses, unlike previous qualitative ELISA analyses in which high background titres were routinely observed, showed that C57BL/6 mice consistently produced low concentrations of anti-TMEV antibody (20–150 μg/ml). In contrast, SJL/J mice consistently exhibited significantly greater antibody responses (600–700 μg/ml). Responses of intermediately susceptible C3H/HeJ mice varied between either high (700 μg/ml—Fig. 1a) or relatively low (250 μg/ml, Fig. 1b) anti-TMEV antibody levels comparable to those seen in C57BL/6 mice.

Virus-specific IgG subclass responses of susceptible and resistant strains of mice to TMEV infection

Although total anti-TMEV levels may predict disease susceptibility, they do little to indicate the underlying mechanisms of the disease in SJL/J, C3H/HeJ and C57BL/6 mice. Analysis of antivirus isotypes revealed that in susceptible SJL/J mice, between Days 29 and 108 post-infection, between 50 and 75% of the anti-TMEV antibody is IgG2a (Fig. 2a). IgG1 and IgG2b each comprise only 20–30% of the response. A similar, yet much more dramatic, antivirus IgG subclass profile is seen in intermediately susceptible C3H/HeJ mice (Fig. 2b). At all time-points assayed approximately 70% of the anti-TMEV response is IgG2a, with IgG1 and IgG2b each comprising only 10–20% of the response. In contrast, in resistant C57BL/6, which display significantly lower levels of total anti-TMEV antibody, IgG1 predominates (80–90%) until Day 39 post-infection, with antibody levels becoming low and thus, extremely variable among individual mice after Day 78 (Fig. 2c). Antivirus IgM levels were barely detectable at the time-points assayed and are not shown. It is unlikely that the isotypic differences between susceptible and resistant mice merely reflect the level of total antivirus antibody produced. C3H/HeJ mice showing lower total antibody responses ranging from 120 to 150 μg/ml (Fig. 1b, Exp. 2), similar to those routinely seen in resistant C57BL/6 mice, displayed the same IgG2a predominance (data not shown) as that found when their total antivirus antibody levels ranged between 600 and 750 μg/ml (Days 78 and 108 post-infection, Fig. 2b). Similarly, C57BL/6 mice maintained an IgG1 predominance whether they showed a very low total anti-TMEV level (50–100 μg/ml, Fig. 2c) or a somewhat higher level (150–200 μg/ml, Fig. 1b) (data not shown). No definite trend in either total anti-TMEV levels or IgG subclass predominance between clinically well and clinically sick C3H/HeJ mice was apparent due to the limited sample size in these experiments (10 mice/group). However, we are currently examining this question using considerably larger sample sizes and additional inbred strains.

Virus-specific IgG subclass responses of susceptible and resistant strains of mice to multiple TMEV immunizations

To determine whether any of these three strains of mice were defective in their ability to produce particular IgG subclasses,

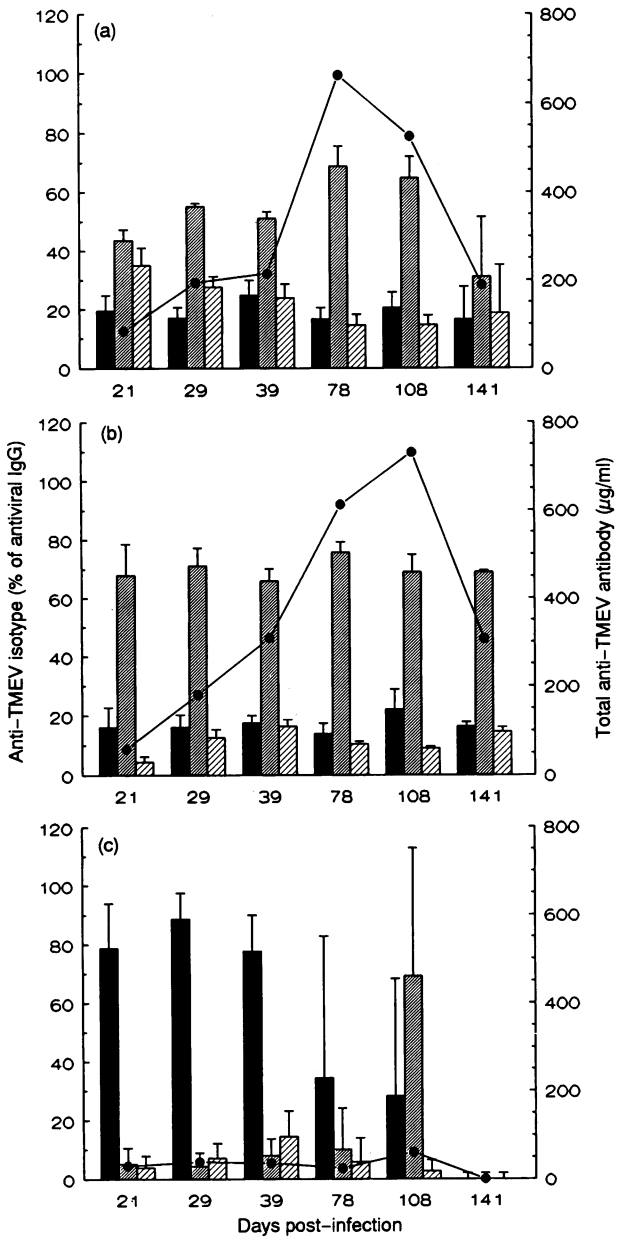

Figure 2. Virus-specific IgG subclass responses of susceptible and resistant strains of mice to TMEV infection. Individual TMEV-infected SJL/J (a), C3H/HeJ (b) and C57BL/6 (c) mice from Exp. 1 (see Fig. 1) were analysed for anti-TMEV IgG subclass concentrations [IgG1 (■), IgG2a (▩) and IgG2b (▨)] at six time-points post-infection. Data are expressed as the mean percentage of each IgG subclass ± SEM composing the total antivirus IgG. Total antivirus antibody concentration (μg/ml) at each time-point after infection (●). Error bars of the total antibody data are omitted for clarity, but are represented in Fig. 1a.

mice were immunized s.c. with 25 μg of UV-TMEV in CFA on Day 0, followed by intraperitoneal immunization with 25 μg of UV-TMEV in Maalox on Days 14 and 21. Sera were collected 14, 21, 28, 51 and 81 days after the primary immunization. Except for the higher antibody levels in SJL/J mice on Day 21 and C57BL/6 mice on Day 51, the total anti-TMEV responses of these three mouse strains did not significantly differ, with all mice achieving a maximal response of 2–3 mg/ml 1 week

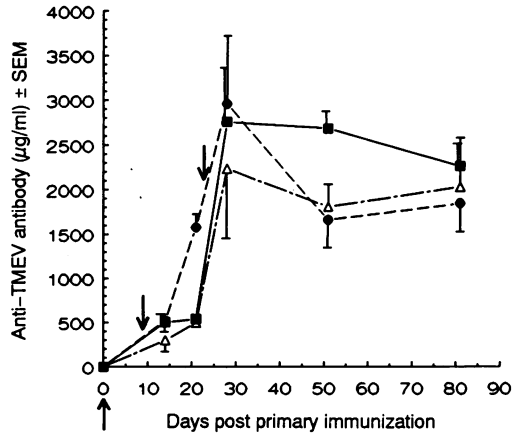

Figure 3. Total anti-TMEV antibody levels in TMEV-immunized SJL/J (●), C3H/HeJ (△) and C57BL/6 (■) mice. Groups of 10 SJL/J, C3H/HeJ and C57BL/6 mice were immunized with 25 μg of UV-inactivated TMEV/CFA s.c. on Day 0 and with 25 μg of UV-TMEV in Maalox i.p. on Days 7 and 20 (arrows). Unimmunized mice showed no TMEV-specific antibody responses (data not shown). The mean total anti-TMEV antibody responses of individual sera were measured by PCFIA at Days 14, 21, 28, 51 and 81 and are expressed in μg/ml ± SEM.

following the final immunization (Fig. 3). The anti-TMEV IgG subclass profiles show that SJL/J and C57BL/6 mice produce equivalent concentrations of IgG1, IgG2a and IgG2b (Fig. 4a, c). C3H/HeJ mice are also able to produce high concentrations of all three of these virus-specific IgG subclasses, however they show a strong preference toward IgG2a antibody production up to 2 months after the final immunization (Fig. 4b).

The anti-TMEV IgG subclass profiles of resistant C57BL/6 and susceptible SJL/J mice following immunization suggest that they are not defective in their abilities to produce high concentrations of anti-TMEV IgG1, IgG2a and IgG2b, yet upon infection these mice produced dramatically different anti-TMEV IgG subclass profiles, with an IgG1 predominance in C57BL/6 mice and an IgG2a predominance in SJL/J mice. In contrast, intermediate C3H/HeJ mice show a strong predominance of anti-TMEV IgG2a following either immunization or infection. This predominance in the percentage of antivirus antibody of the IgG2a subclass in C3H/HeJ mice is not due to excessive production of IgG2a (concentrations are similar to those found in both SJL/J and C57BL/6 mice immunized with TMEV); rather C3H/HeJ mice appear to be slightly deficient in anti-TMEV IgG1 and IgG2b production. We are currently examining if this anomaly is a general feature of antibody responses in C3H/HeJ mice.

DISCUSSION

Virus-specific antibodies can be detected in the serum and CNS of experimental animals with immune- and non-immune-mediated virus-induced demyelinating diseases,[22-27] yet this antivirus antibody does not appear to protect against persistent CNS infection and chronic demyelination. Indeed, antivirus antibody titres have not correlated well with susceptibility to TMEV-induced disease. Although susceptible mice have previously demonstrated slightly higher titres than resistant mice, *both* resistant and susceptible strains of mice showed relatively high anti-TMEV titres as determined by ELISA.[5,7] The apparent

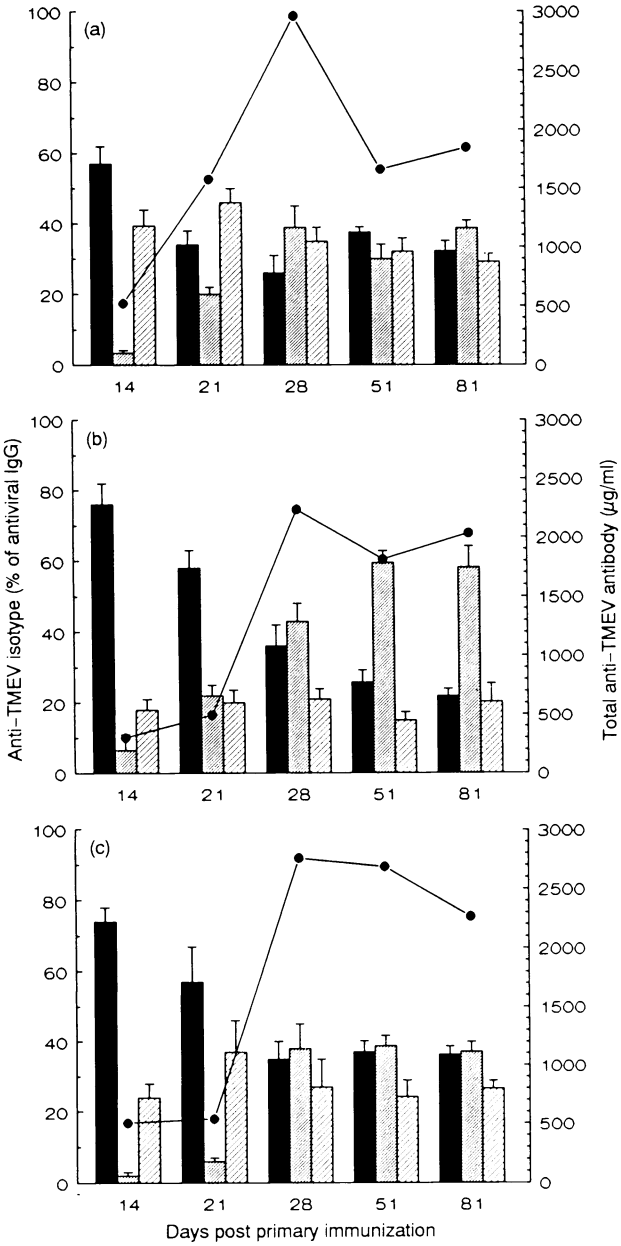

Figure 4. TMEV-specific IgG subclass responses of susceptible and resistant strains of mice to TMEV immunization. Individual TMEV-immunized SJL/J (a), C3H/HeJ (b) and C57BL/6 (c) mice from Fig. 3 were assayed for anti-TMEV IgG subclass responses [IgG1 (■), IgG2a (▨) and IgG2b (▨)] by PCFIA at Days 14, 21, 28, 51 and 81. Data are expressed as the mean percentage of each IgG subclass ± SEM within the total antivirus IgG. Total antivirus antibody concentration (μg/ml) at each time-point following initial immunization (●). Error bars of the total antibody data are omitted for clarity, but are represented in Fig. 3.

ineffectiveness of antibody in combating persistent CNS infection raises the question about whether antivirus antibodies play a role in disease pathogenesis, such as through direct demyelination by mechanisms suggested in human Guillain–Barré syndrome,[28 31] SLE[32] and in canine distemper encephalomyelitis.[25]. Although the pathogenesis of CNS damage in TMEV-induced demyelinating disease appears to be primarily CD4+ T-cell-mediated and virus specific,[5-7] an important secondary role for antibody-mediated effector mechanisms is not excluded. Our initial approach to address the potential contributions of anti-TMEV antibody to disease pathogenesis was through the quantitation of antivirus IgG subclasses produced in TMEV-susceptible and resistant mouse strains.

The present results indicate a preferential production of serum antivirus antibodies of the IgG2a isotype by TMEV-susceptible (SJL/J) and intermediately susceptible (C3H/HeJ) mice following i.c. infection with the BeAn strain of TMEV. In contrast, in TMEV-resistant C57BL/6 mice IgG1 antivirus antibody predominates. This observation is supported by previous results showing high IgG2a/IgG1 ratios in CNS lesions of TMEV infected mice.[24] Murine CD4+ T cells of the Th1 subset mediate DTH and regulate IgG2a production via IFN-γ production, whereas CD4+ Th2 cells regulate IgG1 and IgE production via IL-4.[11 13] It is thus interesting to speculate that the predominant IgG2a antivirus response in susceptible mice correlates with disease susceptibility and is an *in vivo* measure of the preferential stimulation of a Th1-like pattern of cytokine synthesis. Likewise the predominant IgG1 antibody response in resistant C57BL/6 mice may be an *in vivo* measure of preferential stimulation of a Th2 response. In fact, this hypothesis is supported by two additional observations. First, development of clinical disease in SJL/J mice and other highly susceptible mouse strains correlates with the temporal development of high levels of TMEV-specific, MHC class II-restricted DTH which remains elevated for at least 6 months following virus infection. We have proposed that this DTH reactivity against TMEV virions persisting in the CNS white matter leads to macrophage-mediated bystander demyelination.[5,6] In contrast, DTH reactivity is low following TMEV infection of C57BL/6 and other TMEV-resistant mouse strains.[5 7] TMEV infection of intermediately susceptible C3H/HeJ mice leads to high levels of DTH prior to and concomitant with the onset of clinical disease (J. D. Peterson, G. Waltenbaugh and S. D. Miller, unpublished results), which decreases at later times.[7] Second, recent results indicate that CD4+ T cells from susceptible SJL/J, but not resistant C57BL/6, mice make high levels of IFN-γ in response to *in vitro* challenge with TMEV (J. D. Peterson, C. Waltenbaugh and S. D. Miller, unpublished results).

The majority of evidence supporting a different role for Th1- and Th2-derived lymphokines in regulating mouse antibody isotype responses has resulted from *in vitro* studies utilizing T-cell clones.[13] Th1-secreted IFN-γ and Th2-secreted IL-4/IL-10 have been suggested to regulate reciprocally the T cells that produce them as well as regulating IgG subclass production.[11,13] IFN-γ has been shown to induce selectively IgG2a production, suppressing expression of other IgG subclasses, whereas IL-4 selectively induces IgG1 and IgE and inhibits IgG2a production.[11] Similarly, Th2-derived IL-10 inhibits IFN-γ production by Th1 clones.[33,34] There is also evidence of an *in vivo* role for IFN-γ and IL-4 in isotype regulation.[12,35-37] The possibility of selective activation of Th1 and Th2 cells following TMEV infection of susceptible and resistant strains, respectively, is supported by a variety of recent experimental evidence indicating selective activation of different CD4+ T-cell subsets following infection with particular bacterial, protozoan, and virus pathogens depending on the genetics of the mouse host.[14-19] For example, the response to *Leishmania* infection is characterized by IFN-γ production in resistant C57BL/6 mice, but

dominated by IL-4 production in susceptible BALB/c mice.[14,15] Perhaps more relevant to the present system wherein both susceptible SJL/J (H-2^s) and resistant C57BL/6 (H-2^b) mice respond to a determinant(s) mapping within the VP2 capsid protein,[38] it has been shown that the response to a peptide of human type IV collagen is dominated by IFN-γ production in I-A^s mice and by IL-4 production in H-2^b mice.[16,17]

The pattern of antivirus IgG subclass production may thus be predictive of susceptibility or resistance to TMEV-induced demyelinating disease as the increased IgG2a/IgG1 ratio in susceptible strains would reflect their increased DTH (i.e. Th1) activity.[5,7] The possibility remains that Th1-directed IgG2a production may potentiate DTH-mediated demyelination or breakdown of the blood–barrier via its ability to mediate ADCC or activate complement. In mice, IgG2a is more efficient than the other IgG subclass at promoting macrophage phagocytosis (opsonization), activating complement and sensitizing cells for ADCC.[39,40] Production of high levels of antivirus IgG2a antibodies in susceptible mice may facilitate opening of the blood–brain barrier allowing inflammatory T cells access to the CNS. Virus capsid antigens have been demonstrated on the surface of cultured oligodendrocytes in TMEV-induced demyelinating disease,[41] providing a potential target for antivirus IgG2a antibodies. Furthermore, in susceptible SJL/J mice, we have observed (J. D. Peterson, unpublished results) distinct differences in both virus capsid protein specificity and in neutralizing ability between the different IgG subclasses which may contribute to disease susceptibility. In contrast, resistant mouse strains may avoid demyelination through both the absence of DTH responses and antivirus IgG2a and/or through production of higher relative amounts of antivirus IgM[42] or IgG1 (Fig. 1) antibodies which may be more efficient at virus neutralization in C57BL/6 mice. Interestingly, we found that antivirus IgM levels at Day 14 and later were extremely low in both susceptible and resistant strains and varied greatly between individual animals.

Previously, elevated IgG2a/IgG1 ratios have been observed in CBA/RiJ, C57BL/6, and 129/Sv mice following intranasal infection by a variety of viruses, including TMEV. This observation was interpreted as suggesting that either the biochemical nature of virus antigens or the process of infection itself influenced IgG subclass expression.[43] The current observation that TMEV-infected resistant C57BL/6 mice exhibit elevated IgG1 and very low IgG2a responses indicates that high IgG2a/IgG1 ratios cannot be solely the result of the biochemical characteristics of virus antigens. Indeed, this conclusion is further supported by the experiments in which SJL/J and C57BL/6 mice were hyperimmunized via s.c. injection of UV-inactivated virus in CFA. Unlike the distinct DTH levels,[5,7] total anti-TMEV antibody titres (Fig. 1), and anti-TMEV isotype patterns (Fig. 2) observed in these strains following TMEV infection, peripherally immunized mice of both strains produced equally high levels of virus-specific DTH,[7] total anti-TMEV (Fig. 3) and equivalent levels of virus-specific antibodies of the IgG1, IgG2a and IgG2b isotypes (Fig. 4). Thus, the infectious process itself, perhaps due to differences in antigen presentation following CNS infection versus peripheral immunization in adjuvant, or the nature of the antigenic stimulus (viable virus versus UV-inactivated virus) is in part responsible for determining the IgG subclass expression in response to TMEV. In this regard, it has recently become apparent that lymphokine production is influenced by several factors including host genetics,[14–17,19] the nature or form of the antigenic stimulus[44,45] and the type of antigen-presenting cell involved.[46,47] It is apparent that the careful analysis of the types of immune responses and cytokine profiles produced in response to infectious microbes can provide important clues as to the pathogenesis of infectious diseases. We are currently analysing a large panel of TMEV-susceptible and resistant mice for anti-TMEV IgG subclass responses, as well as for virus capsid protein specificity, to provide further insight into the possible role of antivirus antibodies in disease pathogenesis.

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