Short paper

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Genetic control of anti-idiotypic vaccination against coronavirus infection

The idiotypic network can be experimentally altered to induce protective immune responses against microbial pathogens. Both internal image and noninternal image anti-idiotypic (anti-Id) antibodies have been shown to trigger antigen (Ag)-specific immune responses. Therefore, mechanisms of anti-Id vaccination appear to go beyond structural mimicry of Ag, but remain undefined. Using the neurotropic murine coronavirus animal model, we have previously shown that a polyclonal noninternal image anti-Id (Ab2) could vaccinate BALB/c mice. To characterize its mode of action, we have examined the immune modulating capability of this Ab2 *in vivo* in strains of mice with different H-2 haplotypes. Even though only internal image anti-Id are expected to induce non-genetically restricted immunity, this noninternal image Ab2 induced protective immunity in four of eight genetically different strains of mice susceptible to coronavirus infection. These were BALB/c (H-2^d), DBA/2 (H-2^d), DBA/1 (H-2^q), and SWR (H-2^q) mice. Protection was generally correlated with the induction of specific antiviral Ab (Ab3) that showed biological properties, such as virus neutralization *in vitro*, similar to the initial Ab1. To evaluate the genetic implication of the H-2 haplotypes in protection, congenic mice were also tested. Vaccination profiles suggest that cooperation between background gene(s) of the BALB/c mouse with H-2^d and H-2^q loci is necessary for an optimal protective immune response, although the main genetic element(s) regulating the antiviral response to Ab2 inoculation appeared to be located outside the major histocompatibility complex. These results are consistent with the ability of Ab2 to induce protective antiviral antibodies in genetically different animals by biological mimicry.

1 Introduction

The use of anti-idiotypic antibodies (anti-Id Ab) as potential vaccines against infectious agents has been of interest because of the capacity of these Ab to act as surrogate antigens (Ag). Anti-Id are classified into three major categories: $Ab2_\alpha$, $Ab2_\beta$ and $Ab2_\gamma$. The $Ab2_\gamma$ and $Ab2_\alpha$ recognize framework idiotopes either near or distant from the paratope of the Ab1, respectively, whereas $Ab2_\beta$, also called internal image anti-Id, recognize paratope-associated idiotopes. By their potential to molecularly mimic the Ag, $Ab2_\beta$ have been shown to induce neutralizing Ab, protective Ab, or both against various infectious agents [1–3] and to activate T lymphocyte [4]. A true internal image would not be genetically restricted in its ability to induce an immune response, and would be efficient across species barriers [5, 6].

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Abbreviations: **MHV:** Murine hepatitis virus **NRIg:** Normal rabbit immunoglobulins **anti-Id:** Anti-idiotypic antibodies **mAb1:** Monoclonal antibody **Ab2:** Anti-Id **Ab3:** Anti-anti-Id

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Theoretically, only internal image anti-Id were expected to act as surrogate Ag. It was therefore surprising that noninternal image anti-Id induced neutralizing, protective, or both immune responses against a pathogen [7–9]. However, in contrast to immunization with internal image $Ab2_\beta$, non-internal image anti-Id may be genetically restricted by their inability to induce an immune response in different species [10]. Recent studies have also shown that a T cell response could be elicited by noninternal image anti-Id [11, 12]. These observations emphasize the complexity of the idiotypic network and the need to characterize the mechanisms of action of Ab2.

Neurotropic murine coronaviruses such as MHV-A59 induce multiple sclerosis-like diseases in rodents and provide an excellent animal model for studies of the idiotypic network in the context of a virus infection. The viral surface S glycoprotein is the target of humoral [13] and cellular immune responses [14, 15], bears the majority of virus neutralization epitopes [16, 17], and is an important determinant of virulence in animals [18, 19].

We have previously reported the induction of a protective anti-coronavirus immune response in BALB/c mice by polyclonal Ab2 produced in rabbits immunized with a neutralizing and protective mAb1 designated 7–10A [20]. Since the Ab2 preparation did not contain detectable internal image activity and inhibited the interaction between Ab1 and virus, this Ab2 preparation was identified as $Ab2_\gamma$ [20].

To characterize further the protective effect of these Ab2, we asked whether their vaccinating potential could be

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reproduced in coronavirus-susceptible mice belonging to different inbred strains. Our results indicate that the Ab2-induced production of specific anti-coronavirus neutralizing Ab3 is successful only in certain strains of mice. Data obtained with MHC-congenic mice support the conclusion that both H-2 and non-H-2 dependent genes are determining factors in success or failure of anti-coronavirus vaccination with Ab2.

2 Materials and methods

2.1 Animals

New Zealand white female rabbits of 2.5–3 kg were purchased from the *Ferme de sélection Cunipur*, Stukely Sud, Québec, Canada. Mouse hepatitis virus (MHV)-seronegative mice, 4–5 weeks old, were purchased from Charles River, St-Constant, Québec, Canada [BALB/c, DBA/2, C3H, C57BL/6 (B6)], the Jackson Laboratories, Bar Harbor, ME (DBA/1, CBA, BALB.K, C57BL/10 [B10]), or Harlan UK Ltd., Shaw's Farm, Blackthorn, Bicester, GB (B10.D2, SWR, B10.G and BALB.B).

2.2 Virus and cells

Strain A59 of mouse hepatitis virus (MHV-A59) was originally obtained from the American Type Culture Collection (Rockville, MD), plaque-purified twice, and passaged four times at a multiplicity of infection of 0.01 on DBT cells as described [21].

2.3 Anti-Id antibodies and Ab2-induced protective immune response

The immunization protocol for generating anti-Id anti-7-10A, their purification and characterization, as well as the Ab2 immunization protocol, the protection assays, the ELISA and virus neutralization assays for detection of Ab3 against MHV-A59 were performed as described [20].

2.4 Statistics

Results of protection assays *in vivo* were analyzed with the Kaplan-Meier survival curve procedure [22]. Cox's proportional hazards model was used to analyze the reduction in mortality associated with the administration of the anti-Id compared to NRIg [22]. Antiviral Ab3 antibody responses were evaluated and analyzed with the Mann-Whitney test [22]. In these tests, a p value of < 0.05 was accepted as statistically significant.

3 Results and discussion

3.1 Immunization of mice of different strains with Ab2

We have previously shown that rabbit polyclonal anti-Id anti-7-10A (Ab2) were able to induce a protective immune response in BALB/c mice against MHV-A59 [20]. To examine whether the vaccinating property of these Ab2 was genetically controlled, mice of eight different inbred strains were immunized with Ab2 or control normal rabbit immunoglobulins (NRIg) and challenged intracerebrally with ten LD_{50} of MHV-A59. The LD_{50} values ranged between 1.75×10^4 and 8×10^4 PFU, except for B6 and SWR strains, where it was around 6×10^3, and B10.D2 and B10.G mice where it was 1×10^5–1.3×10^5 (data not shown). In agreement with our previous observations [20], about 75% of Ab2-immunized BALB/c (H-2^d) mice survived over 60 days after virus challenge (Fig. 1). However, marked variations in the survival rates were detected from one mouse strain to another. An almost complete protection was obtained in SWR and DBA/1 (H-2^q) mice, whereas only 25–30% of immunized DBA/2 (H-2^d) and CBA (H-2^k) mice survived virus challenge. However, the protection observed in CBA mice was not statistically significant. Moreover, Ab2 had no vaccinating capacity in B6 and B10 (H-2^b) mice, and an apparent reduced survival was even seen in C3H (H-2^k) mice in comparison with control NRIg-treated mice. Thus, Ab2-mediated protection was seen in H-2^q and H-2^d mice, but not in H-2^k and H-2^b mice. These results indicate that the efficacy of Ab2 treatment is genetically controlled and suggest that it may be H-2dependent.

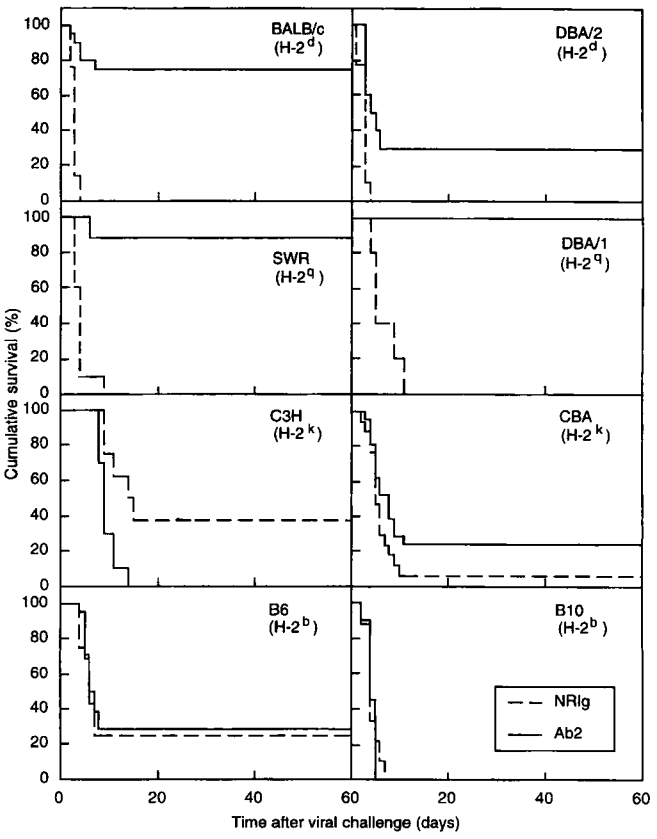

Figure 1. Effect of vaccination with Ab2 on survival of mice of different inbred strains to infection with MHV-A59. Groups of ten mice of different inbred strains were immunized with polyclonal Ab2 or NRIg as described in Sect. 2.3, and challenged with ten LD_{50} of MHV-A59. Their survival was analyzed by the Kaplan-Meier survival curve procedure, as described in Sect. 2.4. Comparison of Ab2 and NRIg treatment by Cox's proportional hazards model showed that the reduction in mortality for BALB/c and DBA/1 mice was significant at $p < 0.001$, DBA/2 at $p < 0.05$ and SWR at $p < 0.01$. The observed increased mortality for C3H mice was significant at $p < 0.05$.

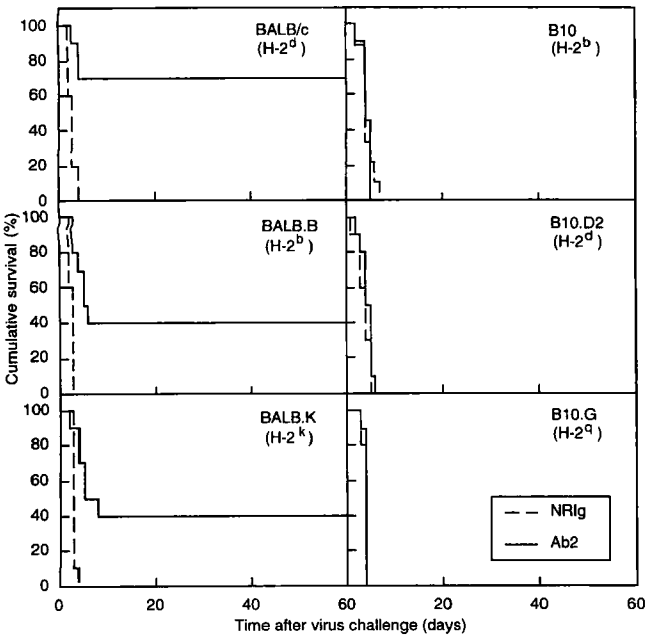

Figure 2. Influence of H-2 or H-2-linked genes on survival of MHV-A59 infected Ab2-immunized mice. Groups of ten MHC-congenic mice on BALB/c and B10 backgrounds were immunized, challenged, and evaluated as described in the legend to Fig. 1. Comparison of Ab2 and NRIg treatment by Cox's proportional hazards model showed that the reduction in mortality for BALB/c mice was significant at $p < 0.001$ and for BALB.B and BALB.K mice at $p < 0.01$.

To investigate further the putative involvement of H-2 or H-2-linked genes in regulating Ab2-mediated protection, MHC-congenic mice on the BALB/c and B10 backgrounds were used. According to survival rates shown in Fig. 1, reduced survival would be expected in BALB.B ($H\text{-}2^b$) and BALB.K ($H\text{-}2^k$) mice should H-2 or H-2-linked genes be involved. Conversely, introduction of the $H\text{-}2^d$ or $H\text{-}2^q$ haplotypes in mice of the B10 background may reverse their resistance to Ab2-induced protection against MHV-A59 infection. Results of these experiments are reported in Fig. 2. As expected, reduction in survival was observed in Ab2-immunized BALB/c congenic mice expressing the $H\text{-}2^b$ or $H\text{-}2^k$ haplotype. However, the introduction of $H\text{-}2^q$ or $H\text{-}2^d$ haplotype in mice of B10 background was not sufficient to override the failure of Ab2 treatment to protect B10 mice from virus challenge (Fig. 2). These results suggest that the capacity of Ab2 to protect mice against MHV-A59 infection is mainly dependent on genetic elements located outside the MHC complex. However, given an appropriate genetic background, the amplitude of the Ab2-induced antiviral response would probably be regulated by H-2 or H-2-linked gene(s). This conclusion is supported by the observed variations in survival in MHC-congenic mice on the BALB/c background. It remains to be elucidated whether a unique background gene shared by BALB/c, DBA/1, DBA/2, and SWR mice is responsible for the capacity of these mice to respond to Ab2 anti-7-10A. This is, however, unlikely since mice of these four strains were not protected to the same level following Ab2 vaccination. Further experiments are needed before the genes involved are identified. Testing the Ab2-mediated anti-coronavirus protection in CXB recombinant inbred mice may help to characterize the BALB/c-associated gene.

3.2 Induction of antiviral antibodies in Ab2-immunized mice

To evaluate whether variations in Ab2-mediated protection of mice belonging to diffeent strains correlated with the presence of antiviral antibodies (Ab3), the sera of immunized mice were tested by ELISA and virus neutralization assays for the presence of MHV-A59-specific Ab3. The results presented in Table 1 indicate that mice of any

Table 1. Induction of specific and neutralizing anti-MHV-A59 Ab3 in protected Ab2-immunized mice

Mouse strain	H-2 haplotype	No. of mice per treatment[a)]	Corrected ELISA absorbance at 1/500 serum dilution[b)]	Significance of ELISA absorbance value[c)]	Neutralization titer[b)]
BALB/c	d	20	0.96 ± 0.25	< 0.0001	100–675
DBA/2	d	10	0.17 ± 0.20	NS[d)]	< 50–125
B10.D2	d	10	0.20 ± 0.15	NS[e)]	< 50
SWR	q	10	0.93 ± 0.53	0.0075	< 50–725
DBA/1	q	6	0.75 ± 0.10	0.0039	500–3000
B10.G	q	9	0.25 ± 0.15	NS	< 50
C3H	k	10	0.00 ± 0.01	NS	< 50
CBA	k	6	0.02 ± 0.02	NS	< 50
BALB.K	k	10	0.33 ± 0.19	0.0011	< 50–50
B6	b	6	0.02 ± 0.04	NS	< 50
B10	b	11	0.10 ± 0.11	NS	< 50
BALB.B	b	10	0.70 ± 0.41	0.0019	< 50–400

a) Groups of identical sizes were immunized with either Ab2 or NRIg.
b) Sera were assayed by ELISA for binding to virus (absorbance given for Ab2-treated mice; NRIg treatment yielded values that ranged between 0.02 and 0.43) and plaque reduction assays (neutralization titer is the reciprocal of the highest dilution of serum neutralizing 50% of input virus; NRIg treatment yielded titers that were all < 50).
c) p values from Mann-Whitney test comparing absorbance means at 1/500 dilution of sera between Ab2- and NRIg-treated mice.
d) Even though Ab2-protected DBA/2 mice did not show significant ELISA signals, they did produce Ab3 as shown by neutralization titers.
e) Not significant.

strain in which protection was observed after Ab2 treatment developed significant levels of MHV-A59-reactive and neutralizing Ab3. This suggests an overall link between Ab2-induced antiviral Ab3 and the observed protection of mice from viral challenge. This was confirmed by the Cox's proportional hazards statistical model, where the high serological ELISA response of each mouse was defined as a response higher than the median serological scores specific for responding mice of a given strain. For Ab2-protected mouse strains, the reduction in mortality correlated with a high serological response. Indeed, we observed a 78% reduction in mortality at a p value of less than 0.001 for responder strains, whereas no significant reduction of mortality was seen in nonresponder strains (data not shown).

The ability of Ab3 to neutralize virus infectivity suggests that protection against MHV-A59 observed in Ab2-immunized mice may be mostly due to this property of Ab3. However, careful examination of the data allowed us to identify some differences in titers of neutralizing antibodies in the sera of BALB.B and BALB.K mice that showed the same level of Ab2-induced protection (data not shown). These differences could indicate the possible involvement of other components of the immune response in protection. Such studies are in progress.

4 Concluding remarks

Due to their capacity to induce immune response across species barriers, $Ab2_\beta$ remains undoubtedly the anti-Id of interest for vaccination against microbial pathogens. However, the present demonstration that rabbit polyclonal $Ab2_\gamma$ anti-Id can induce a protective immunity against coronavirus infection in mice of unrelated inbred strains supports the idea that noninternal image anti-Id may also be considered as good candidates for vaccination against pathogens.

We have reported elsewhere that unlike the $Ab2_\gamma$ used in the present study, another rabbit $Ab2_\gamma$ was inefficient in vaccinating BALB/c mice against lethal MHV-A59 infection [23]. The reasons why a noninternal image $Ab2_\gamma$ induces a protective immunity, whereas another of the same type raised against a mAb1 with related properties does not, are intriguing. The relative contribution of the different components of the immune network in Ab2-induced vaccination is not yet fully elucidated. It should also be kept in mind that, under certain circumstances, immunization with anti-Id may even be deleterious. Indeed, a significant increase in mortality, rather than protection, was observed in one of the mouse strains (C3H) used in this study. Similarly, Kennedy et al. [24] reported an increase in the pathogenicity of herpes simplex virus following immunization of BALB/c mice with a polyclonal Ab2. Therefore, a better understanding of the interactions between the components of the idiotypic network is certainly needed before anti-Id can be exploited as successful, reproducible and safe alternative vaccines against microbial pathogens.

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