MHC Restriction of V-V Interactions in Serum IgG

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According to Jerne's idiotypic network hypothesis, the adaptive immune system is regulated by interactions between the variable regions of antibodies, B cells, and T cells.[1] The symmetrical immune network theory[2-6] is based on Jerne's hypothesis, and provides a basis for understanding many of the phenomena of adaptive immunity. The theory includes the postulate that the repertoire of serum IgG molecules is regulated by T cells, with the result that IgG molecules express V region determinants that mimic V region determinants present on suppressor T cells. In this paper we describe rapid binding between purified murine serum IgG of H-2^b and H-2^d mice and serum IgG from the same strain and from MHC-matched mice, but not between serum IgG preparations of mice with different MHC genes. We interpret this surprising finding in terms of a model in which IgG molecules are selected to have both anti-anti-self MHC and anti-anti-anti-self MHC specificity.

The symmetrical immune network theory of the regulation of the adaptive immune system[2-6] has been developed since the mid 1970s. The theory is based on Jerne's immune network hypothesis.[1] The theory resolves several paradoxes and makes experimentally testable predictions.[6] One of the predictions is that serum IgG is a quasi-species, meaning that IgG molecules have V regions that are similar to each other. The theory also specifies that V regions of IgG antibodies mimic determinants that are present on suppressor T cells. In this paper we report MHC restriction of V-V interactions in serum IgG of mice, which provides experimental support for this aspect of the theory.

A recurring theme in the symmetrical network theory is co-selection.[5] For example, suppressor T cells are a central regulating element of the system, and they are co-selected with helper T cells. Co-selection means that the suppressor T cells are selected on the basis of having V regions with complementarity to as many helper T cells as possible, and conversely helper T cells are selected to have complementarity to as many suppressor T cells as possible. An additional constraint on the selection of helper T cells

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is that they are selected such that their V regions have complementarity to self components, including especially self MHC class II.

In the theory IgG secreting clones are also co-selected with helper T cells.[3] This co-selection results in serum IgG being a quasi-species, that expresses anti-anti-self epitopes similar to anti-anti-self epitopes present on suppressor T cells.

IgG was purified from the serum of BALB/cJ, C57BL/6J, B10 and B10.D2 mice. Biotin and horse radish peroxidase (HRP) were coupled to aliquots of the purified IgG to produce BALB/cJ biotin-IgG, C57BL/6J biotin-IgG, B10 biotin-IgG, B10.D2 biotin-IgG, BALB/cJ HRP-IgG, C57BL/6J HRP-IgG, B10 HRP-IgG and B10.D2 HRP-IgG.

Binding of the IgGs to each other was measured in ELISA assays using avidin-coated plates. Various concentrations of biotin-coupled IgG were incubated on the plates and unbound IgG was washed off with PBS. HRP-coupled IgG was then incubated on the plates for 30 min, 1, 3 or 18 h, and unbound IgG was washed off with PBS. An HRP substrate was added, the plates were incubated at room temperature, then the changes in optical density were determined. The results shown in the figures are the mean and standard deviation for eight-fold replicas.

Results for binding times of 30 minutes, 1 hour, 3 hours and 18 hours for the binding of BALB/c IgG on BALB/c IgG, BALB/c on C57BL/6, C57BL/6 on BALB/c and C57BL/6 on C57BL/6 are shown in Figure 1. The results for 30 minutes and 1 hour show that there is rapid binding of BALB/c IgG on BALB/c IgG and C57BL/6 IgG on C57BL/6 IgG, and no rapid binding of BALB/c IgG on C57BL/6 IgG or vice versa (30 minute and 1 hour time points). On the other hand, at 3 hours and 18 hours binding of BALB/c IgG on C57BL/6 IgG and vice versa emerges.

Additional results for 1 hour binding, that include the MHC congenic strains B10 (H-2^b) and B10.D2 (H-2^d), are shown in Figure 2. IgG from BALB/c (H-2^d) binds rapidly to IgG from B10.D2, but not to IgG from B10, and conversely IgG from C57BL/6 (H-2^b) binds rapidly to B10 but not to B10.D2. Furthermore, IgG from B10 binds rapidly to IgG from B10 but not to IgG from B10.D2, and IgG from B10.D2 binds rapidly to IgG from B10.D2 but not to IgG from B10. These results show that the rapid self-binding phenomenon seen at the 1 hour time point is restricted by genes in the major histocompatability complex.

The major histocompatability complex is known to play a central role in the repertoires of T cells,[7] but has not previously been shown to impact on the repertoires of antibodies. In order to interpret these results we begin by

reviewing some of the basic features of the symmetrical immune network theory. Specific T cell factors (tabs) play a central role in the theory.[2,3,6] Tabs have a molecular weight of about 50,000, and are able to exert potent regulatory effects on the adaptive immune system.[8] There is evidence that tabs are cytophilic for non-specific accessory cells (A cells) including macrophages.[9] When bound to A cell surfaces, antigen-specific tabs are assumed to be able to stimulate antiidiotypic T and B cells, and antiidiotypic tabs are assumed to be able to stimulate antigen-specific T and B cells. A mixture of antigen-specific and antiidiotypic T cells would then be able to stimulate T and B cells that are both antigen-specific and antiidiotypic. T cells are selected to have some complementarity to MHC molecules. The MHC restriction seen in our results is most simply understood in terms of a mixture of anti-MHC and anti-anti-MHC tabs being present on A cells, with this mixture selecting IgG producing B cells that are both anti-anti-MHC and anti-anti-anti-MHC. These IgG molecules are then able to exhibit MHC restricted binding to each other. This is a very rapid process due to the fact that all of the IgG antibodies are selected to have this dual specificity, so that the antibodies quickly find a complementary antibody, in spite of the low concentrations of the antibodies used in the assays.

We ascribe the slower binding between BALB/c IgG and C57BL/6 IgG, that is seen at 3 hours and 18 hours, to the conventional diversity of BALB/c V regions and C57BL/6 V regions, with antibodies needing a longer time to diffuse and encounter by chance antibodies with complementary specificity, independent of any self-specific MHC-restricted epitope or epitopes.

In summary, the finding that IgG exhibits MHC-restricted binding in $H\text{-}2^b$ and $H\text{-}2^d$ mice is compelling evidence of the importance of idiotypic network regulation in the adaptive immune system, and can be understood as outlined here in the context of the symmetrical immune network theory.[6]

METHODS SUMMARY

Animal Care: During this study, the care, housing and use of animals was performed at the Zoology Small Mammal Unit, Department of Zoology;, University of British Columbia (UBC), in accordance with the Canadian Council on Animal Care guidelines. The methodology described here was reviewed and approved by the UBC Committee on Animal Care prior to conducting the studies.

IgG purification: BALB/cJ, C57BL/6J, B10, and B10.D2 mice were sacrificed by CO_2 asphyxiation and whole blood collected by cardiac puncture. Serum was collected by centrifugation at 3000g and IgG collected with the Melon™ Gel IgG

Spin Purification Kit (Pierce, Rockford, Il). Purity was confirmed by Western Blot, and IgG concentration determined by Coomassie Blue staining.

Biotinylated IgG Preparation: Purified IgG was diluted in oxidation buffer (0.1 M sodium acetate buffer, pH 5.5) to 2 mg/mL. One to one cold sodium meta-periodate solution to cold IgG was mixed and the reaction vessel was protected from light and incubated for 30 minutes at 4°C. Excess periodate was removed by gel filtration through a Zebra™ Desalt Spin Column equilibrated with coupling buffer (Pierce.) One to nine parts prepared 50 mM Biotin Hydrazide Solution to 9 parts oxidized and buffer-exchanged sample (results in 5 mM Biotin Hydrazide) was mixed for 2 hours at room temperature. Biotinylated IgG was separated from non-reacted material by Zeba™ Desalt Spin Columns. Biotinylated samples were aliquoted and stored at -70C. The concentrations of the stock solutions were 12.5 mg/mL for BALB/c biotin-IgG and 27 mg/mL for C57BL/6 biotin-IgG. For the experiment of Figure 1 the concentrations of biotin-IgG used were the stock solutions diluted by a factor of 10^8.

HRP-conjugated IgG Preparation: Purified IgG was conjugated at pH 7.2. Purified IgG was diluted in PBS to 2 mg/mL. 10 mg/mL EZ-Link® Plus Activated Peroxidase with 100 µl of ultrapure water was added to the IgG solution. 10 µl of Sodium Cyanoborohydride was immediately added to the reaction and incubated for 1 hour in a fume hood at room temperature. 20 µl of Quenching Buffer was added and reacted at room temperature for 15 minutes. Conjugated samples were aliquoted and stored at -70C. The concentrations of the stock solutions were 19.1 mg/mL for BALB/c HRP-IgG and 29 mg/mL for C57BL/6 HRP-IgG. For the experiment of Figure 1 the concentrations of HRP-IgG used were the stock solutions diluted by factors of 10^6, 10^7, 10^8, 10^9 and 10^{10}.

ELISA Assays: Binding of the IgGs to each other was measured in ELISA assays using avidin-coated plates. Various concentrations of biotin-coupled IgG were incubated on the plates at room temperature for 30 minutes, and excess reagent washed off with PBS for 3 x 5 min. HRP-coupled IgG was incubated on the plates for 30 min, 1, 3 or 18 h and unbound IgG washed off with PBS for 3 x 5 min. 1-Step™ Turbo TMB-ELISA reagent was added and incubated at room temperature for 30 min and stop solution added. Absorbance was read at 450 nm. The results are presented as mean and standard deviation for eight-fold replicas.

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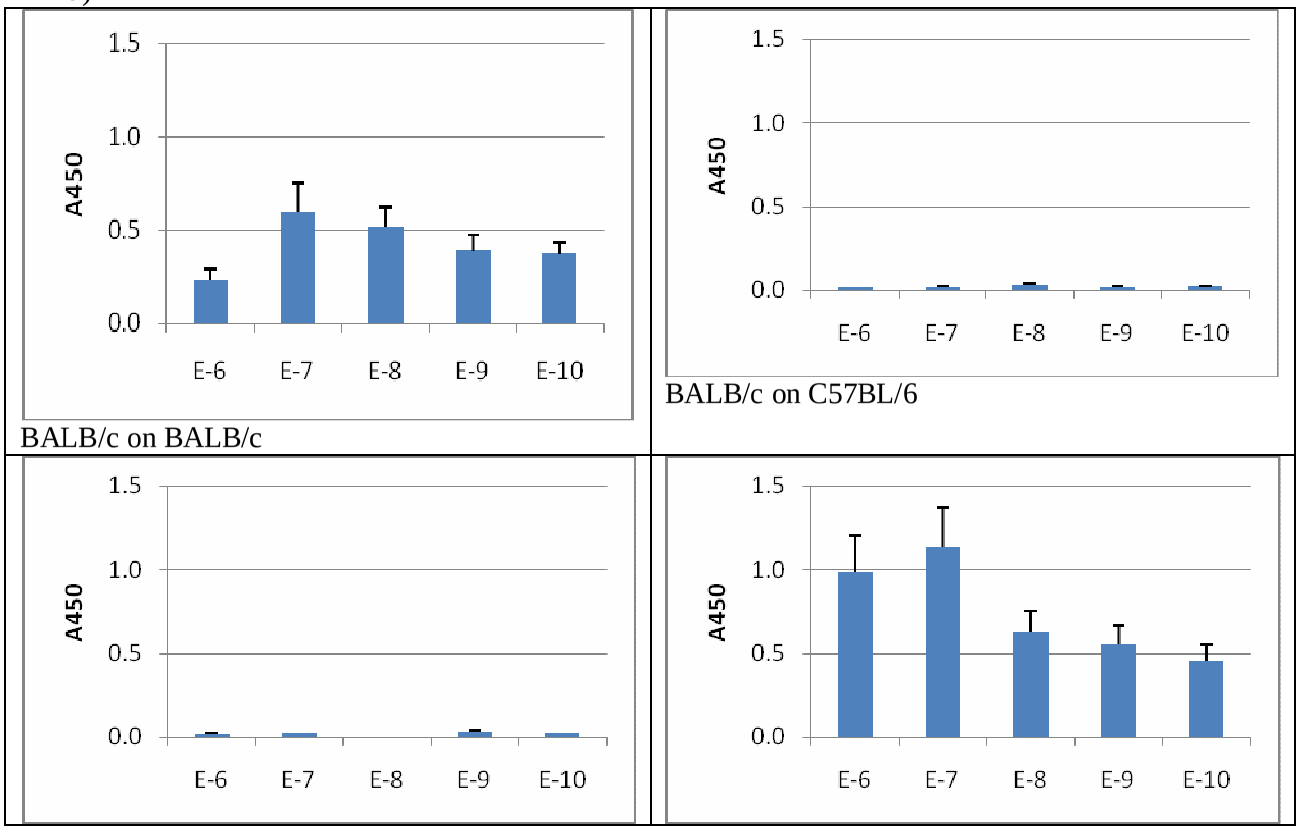
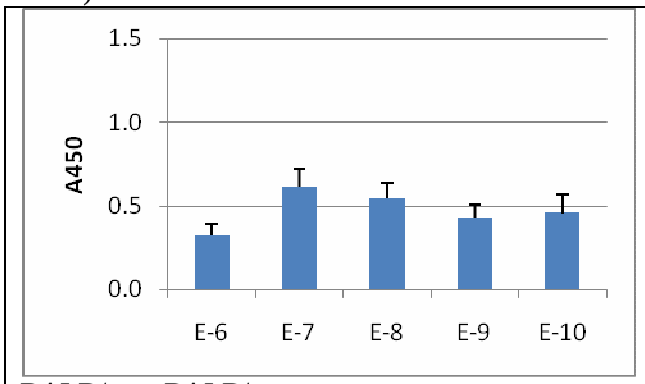
Figure 1. ELISA assay results for the binding of binding of BALB/c serum IgG on BALB/c serum IgG, BALB/c on C57BL/6, C57BL/6 on BALB/c, and C57BL/6 on C57BL/6 at a) 30 minutes b) 1 hour c) 3 hours and d) 18 hours.

a)

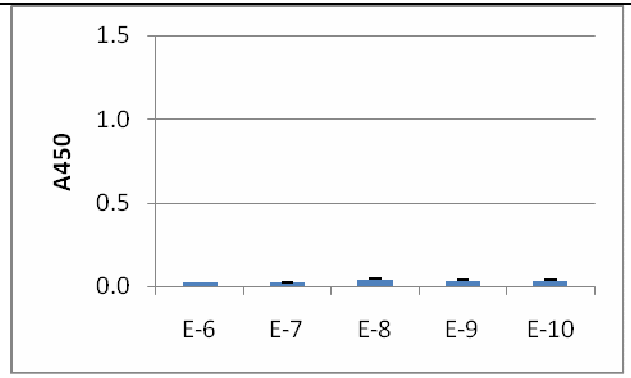


C57BL/6 on BALB/c	C57BL/6 on C57BL/6

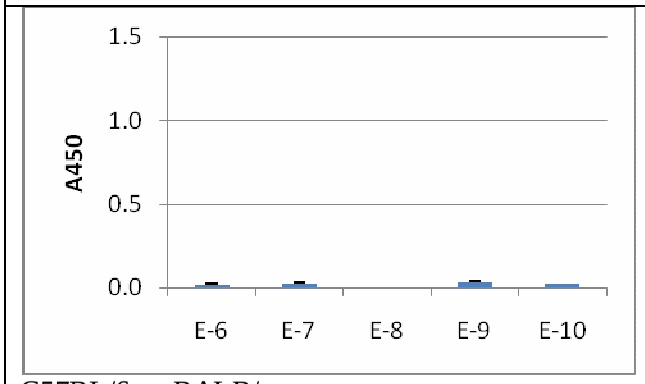
b)



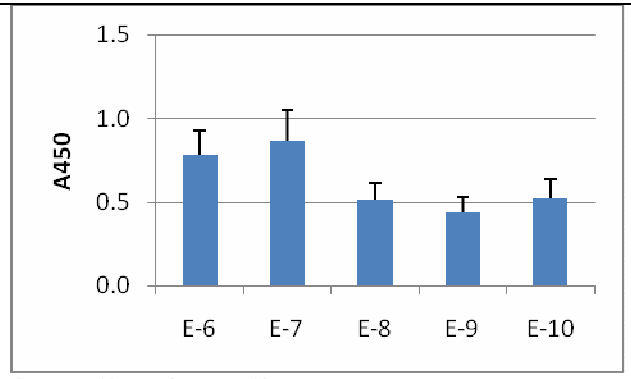
BALB/c on BALB/c



BALB/c on C57BL/6 and

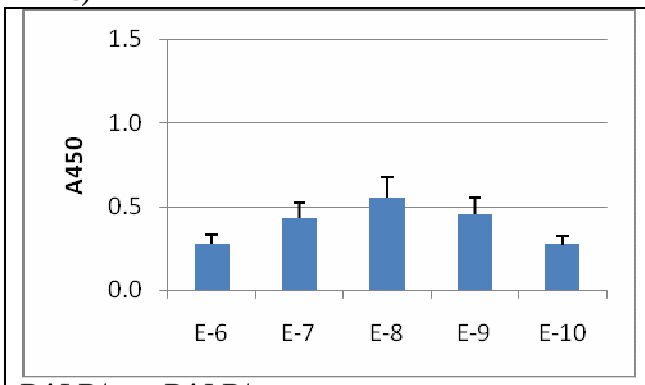


C57BL/6 on BALB/c

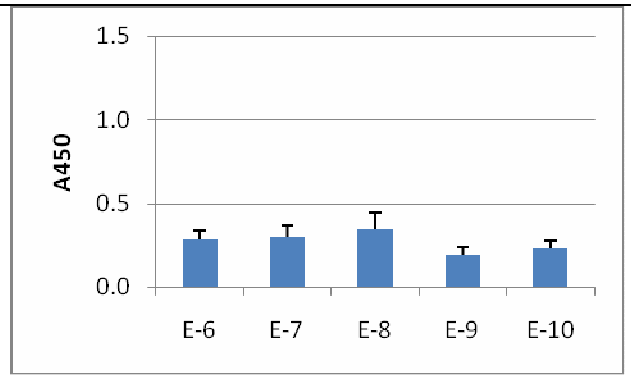


C57BL/6 on C57BL/6

c)



BALB/c on BALB/c



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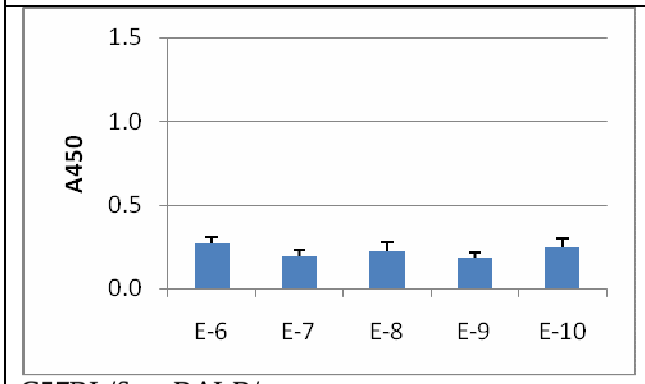

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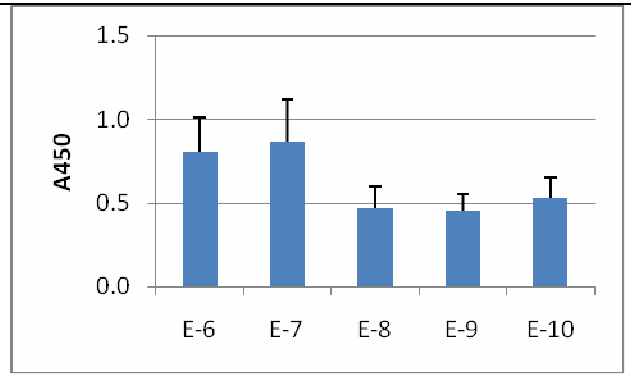
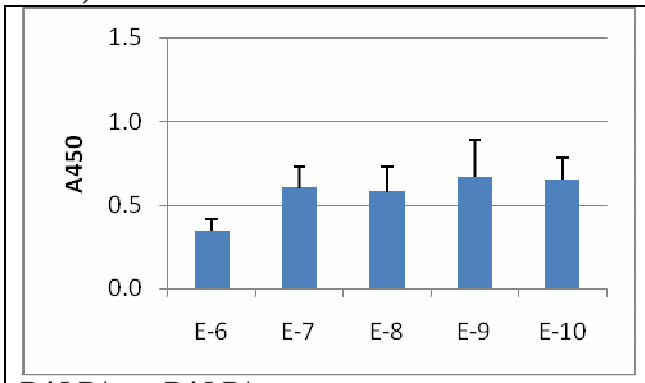
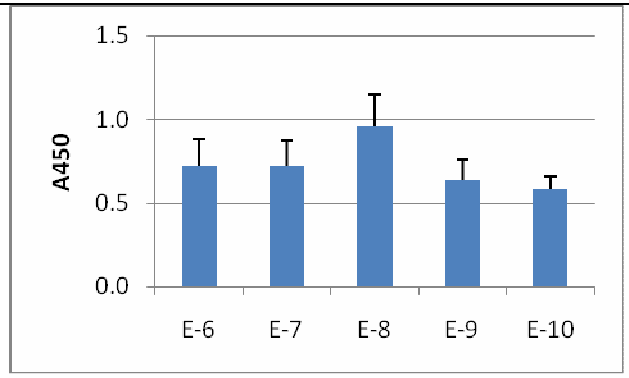


C57BL/6 on C57BL/6

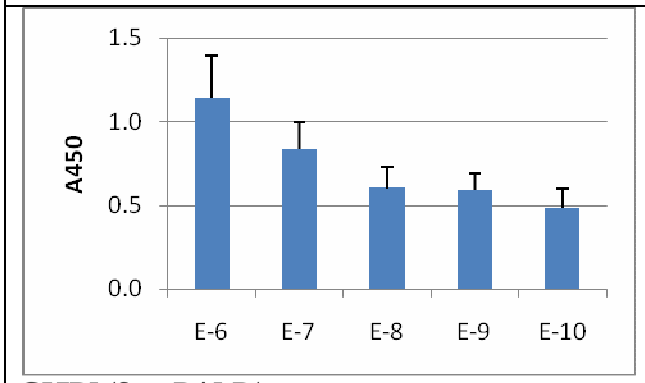
d)



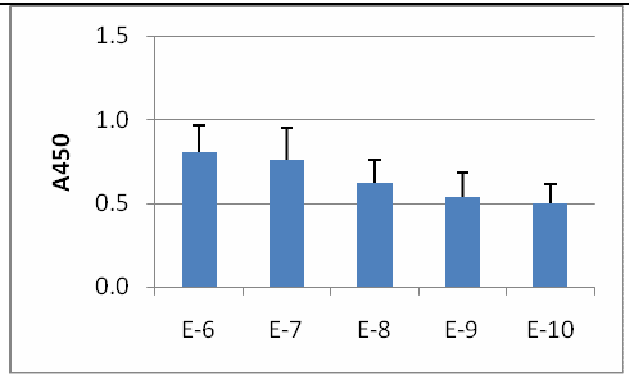
BALB/c on BALB/c



BALB/c on C57BL/6



C57BL/6 on BALB/c



C57BL/6 on C57BL/6

Figure 2. ELISA assay results for the binding at 1 hour of serum IgG to serum IgG involving purified IgG from B10, BALB/c, C57BL/6 and B10.D2 mice.