Cross-Reaction of Anti-Simian Immunodeficiency Virus Envelope Protein Antibodies with Human Immunoglobulins

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Abstract

It has been recently established that retroviral envelope proteins (REPs) have structural features similar to those of immunoglobulins (Igs). In this study, we asked whether anti-REP antibodies cross-react with human Igs (hIgs). To this end, murine monoclonal antibodies (mMoAbs) that had been raised against a simian immunodeficiency virus (SIV) envelope protein, SIVMac251gp120, were screened for their ability to react with human monoclonal Igs (HMIgs). We show that two HMIgs, RFSJ2 (a rheumatoid factor) and PAMLN6 (a human anti-hIg V region antibody), but not a number of other HMIgs, could be weakly, but consistently, bound by anti-SIVMac251gp120 mMoAbs KK17 and KK46, as judged by indirect enzyme-linked immunosorbent assay and a liquid-phase inhibition immunoassay. Both mMoAbs are specific to amino acid residues in the V3 loop of the SIVMac251gp120. The RFSJ2 Ig heavy-chain V region (V_H) is coded in part by a human V_H gene, V_H3–30.3 and includes the idiotope 7B4 (NKYY), which was previously shown to be present in the gp120 protein of a number of HIV-2 and SIV strains. However, an entirely different V_H gene codes the PAMLN6 V_H region, opening the possibility that epitope(s) shared between SIVMac251gp120 and hIgs may not be limited to the 7B4 idiotope.

Introduction

Representatives of the Lentivirinae subgroup of retroviruses cause a progressive failure of the host immunologic functions, thus triggering deadly diseases, such as the acquired immunodeficiency syndrome (AIDS). Although the initial immune response to the infection by these viruses may be vigorous, the function of the immune system almost inevitably collapses at some point, and a fatal outcome often follows [1]. It has been documented that the primate retroviruses, including the human immunodeficiency virus (HIV) and the simian immunodeficiency virus (SIV), paralyse the host immune system by targeting CD4-expressing T lymphocytes and macrophages – cells that are crucial for the emergence and regulation of the effective antiviral immune response [2]. In addition to affecting T lymphocytes and macrophages, more recently it is being documented that HIV and some other retroviruses also strongly affect B lymphocytes and thus alter the humoral immunity [3]. The exact mechanisms of the influence of HIV and other retroviruses on the B-cell compartment of the immune system are being currently investigated.

HIV-infected individuals show signs of polyclonal B-cell activation, weakened specific antibody responses, loss of memory cells, accelerated differentiation into plasma cells, oligoclonal B-cell expansion and changes in the immunoglobulin (Ig) gene repertoire [3–5]. These abnormalities of the humoral immunity may be in part caused by deregulated T cells [6]. However, more recent evidence suggests that B cells themselves may be directly affected by retroviruses. Some, although not all, human B-cell lines, as well as freshly isolated human B lymphocytes, can be productively infected by HIV, perhaps owing to the ability of the virus to bind to a chemokine receptor expressed on these cells [7, 8], or interaction with specific idiotypes. The binding of HIV envelope protein, gp120, to V regions of some Igs has

also been interpreted as having a superantigen-like effect on the B cells of the infected individuals [9].

The ability of gp120 and, possibly, other retroviral envelope proteins (REPs) to bind certain antigenic epitopes, including idiotopes, may be related to the fact that REPs have a number of antibody-like structural properties. REPs are folded into loops that resemble Ig domains, containing three constant (C1–C3) and three variable (V1–V3) domains [10–12]. By computer-assisted amino acid homology search, we have previously shown that certain oligopeptides are shared between the V3 loop of some HIV-2 and SIV isolates, and the tetrapeptide sequences between positions 56 and 59 on the V region of human Igs (hIgs) heavy-chain V regions (V_H) coded by a small group of related Ig V region genes (V_H3–30, V_H3–30.3 and V_H3–33 [13]). In the same study [13], we also reported the existence of similar molecular homologies between the V2 domain of some HIV-1 isolates and the V_H56–59 positions of Ig encoded by a set of nine different Ig V-region genes (V_H1–3, V_H1–18, V_H1–46, V_H1–58, V_H4–4, V_H4–34, V_H4–59 and V_H4–61). Despite the degeneracy of the genetic code, the fact that these homologies exist at the nucleotide level strongly implies that such sequence similarities between hIg and the V-regions of viral gp120 are not coincidental, but that they result from exchange of genetic material between the human and retroviruses. Furthermore, the maintenance of these sequences on the highly variable regions of gp120 suggests that these sequences provide a survival advantage to the virus.

Whether these apparent molecular homologies between REPs and hIg V regions have any functional impact on the immunity of individuals infected by retroviruses is not known. Because of these homologies, antibodies elicited in the host against REPs may cross-react with the host's own Ig V regions. Such cross-reactivity might mean that in primates (including humans) with retroviral infections the sequences of REP proteins homologous to hIg V regions (or anti-REP antibodies) serve as idiotypes (or elicit anti-idiotype antibodies), which disturb the regulation of the immune system by normal idiotypic network interactions [14, 15] and interfere with the functional capabilities of selected B lymphocytes. Such idiotypic disturbance may, at least in part, explain the oligoclonal B-cell expansion frequently observed in HIV-infected humans [3]. Although a speculative possibility, chronic stimulation by cross-reactive anti-REP antibodies of B cells carrying certain idiotypes may also contribute to the development of AIDS-related lymphomas. Yet, it has not been shown so far that antibodies raised against retroviral proteins may indeed react with hIgs, in particular with their V regions.

To address these issues, we screened a panel of murine monoclonal antibodies (mMoAbs) that had been raised against a simian immunodeficiency virus (SIV) envelope protein, SIVMac251gp120, for their ability to react with human monoclonal Igs (HMIgs). We show here that some HMIgs are weakly, but consistently, bound by anti-SIVMac251gp120 mMoAbs, while others are not. Of note, of the two HMIgs bound by an anti-SIVMac251gp120 mMoAbs, one has a V_H3–30 gene-coded V_H region (recognized by the anti-idiotypic antibody 7B4), which has previously been shown to have an amino acid sequence homology with some REPs. The other, however, has an entirely different structure of its V region, suggesting that the cross-reactive epitope, if it exists, may not be identical with the 7B4 idiotope.

Materials and methods

SIV protein. The SIVMac251gp120 protein was purchased from Advanced Biotechnologies (Columbia, MD, USA) (Catalogue Number 14-104-050, Lot Number 111-072) and stored in aliquots at −20 °C before use.

mMoAbs. The mMoAbs of the KK series, specific to known portions of the SIVMac251gp120 molecule and originally generated by Kent *et al.* [16], were kindly provided by the National Institutes of Health AIDS Research and Reference Reagent Program (NIHARRP). Their properties have been described by Kent and her coworkers [16] and summarized in the NIHARRP catalogue [17]. All of the mMoAbs used in this study were of the IgG, κ isotype. The mMoAbs were supplied in small aliquots as partially purified ascites fluids and stored in aliquots at 4 °C before use.

HMIgs. The HMIgs RFSJ2, RFKL1, PRTS2, PAMLN5, PAMLN6 and AH-1 have been characterized previously [18–20]. Their properties, essential for this investigation, are summarized in Table 1. The HMIgs were produced by Epstein–Barr virus-transformed cell lines and partially purified from the cell lines' supernatants by ammonium sulphate precipitation, followed by dialysis against phosphate-buffered saline (PBS), pH 7.3. The concentration of the HMIg in the partially purified samples was determined by sandwich enzyme-linked immunosorbent assay (ELISA), where a human myeloma-derived IgM (Calbiochem, San Diego, CA, USA) was used to build standard curves. Other reagents for the sandwich ELISA were purchased from Biosource International (Camarillo, CA, USA). The HMIgs were stored at 4 °C or in aliquots at −20 °C before use.

Indirect ELISA. This was done, essentially, as described [21]. Briefly, wells of MaxiSorp ELISA plates (Nunc, Aarhus, Denmark) were coated with SIVMac251gp120 or with a HMIg dissolved in PBS (0.1 μg/well) overnight at room temperature (RT) and blocked with 2% nonfat milk and 0.05% Tween-20 (Sigma, St. Louis, MO, USA) in PBS. Dilutions of the mMoAbs were then added and allowed to react with the adsorbed antigen for 1 h at RT. The reactions were developed by adding goat antimouse IgG antibody conjugated with horseradish peroxidase (GAM-POD) at 1 : 2000 for 1 h at RT, and then the solution of 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic

Table 1 Human monoclonal immunoglobulins used in this study

	Class		Variable region				
Name	Heavy chain	Light chain	Heavy chain	Light chain	V_H56-59*	Specificity	Reference
AH-1	IgM	λ	V_H1-18	$V_λ$ 1	Not Known	RF	18, 19
RFKL1	IgM	κ	V_H3-23	$V_κ$ 1	ITYY	RF	18, 19
RFSJ2	IgM	λ	V_H3-30.3	$V_λ$ 1	NKYY	RF	18, 19
PRTS2	IgM	λ	V_H4-34	$V_λ$ 3	STNY	RF	18, 19
PAMLN5	IgM	λ	V_H5-51	Not known	NTKY	Anti-human Fab	20
PAMLN6	IgM	λ	V_H5-51	Not known	DTRY	Anti-human Fab	20

*Amino acid residues at positions 56, 57, 58 and 59. Ig, immunoglobulin.

acid and hydrogen peroxide in citric buffer, pH 4.6, for 10 min. The absorbance in the wells was read at 405 nm (background 655 nm) with the help of the microplate reader 3550-UV (Bio-Rad Laboratories, Hercules, CA, USA).

Inhibition assay. This was done, essentially, as described [21]. Briefly, a fixed amount of a mMoAb was incubated with increasing amounts of HMIg or SIVMac251gp120 overnight at 4 °C. The mixtures were then used as sources of anti-SIVMac251gp120 antibody in the indirect ELISA carried out as described in the above section. In these experiments, only SIVMac251gp120 served as the solid-phase-adsorbed antigen. The results are expressed either as absorbance values or as percentages of inhibition, assuming that these percentages equalled 0% when no HMIg or SIVMac251gp120 was added.

Statistics. To determine the significance of results, Student's *t*-test for small samples was used. Results were considered significant at $P < 0.05$.

Results

Some anti-SIVMac251gp120 mMoAbs bind RFSJ2 in an indirect ELISA

Previously, we have demonstrated that certain oligopeptides are shared between the V3 loop of some HIV-2 and SIV isolates, and tetrapeptide sequences between positions 56 and 59 on the V_H region coded by a small group of related hIg V region genes (V_H3–30, V_H3–30.3 and V_H3–33 [13]). In this study, we initially asked whether mMoAbs specific to the V3 loop of one particular SIV envelope protein, SIVMac251gp120, are able to bind RFSJ2, a HMIg whose V_H region is coded in part by the V_H3–30.3 gene [18, 19]. A panel of mMoAb specific to different portions of the SIVMac251gp120 was screened for this purpose with the help of an indirect ELISA, as described in the *Materials and methods*. The results are shown in Fig. 1. As shown, anti-SIVMac251gp120 mMoAbs, KK46 and KK17 (both specific to the V3 loop of the viral protein), bound the HMIg, albeit weakly (~40–50% of the positive control), while other anti-SIVMac251gp120 mMoAbs, KK8 (anti-V1/V2) and KK45 (anti-V3, nonoverlapping with KK46) showed even weaker binding to the HMIg (less than 30% of the positive control). The anti-SIVMac251gp120 mMoAb, KK65 (anti-V1), also showed a marginal binding to the HMIg (less than 30% of the positive control). In addition, mMoAbs specific to other SIVMac251 envelope proteins, gp41 (KK15 and KK41) and p17Gag (KK59 and KK64) did not bind RFSJ2 (not shown). These results suggested that the mMoAbs KK17 and KK46 could recognize an epitope shared between the SIVMac251gp120 V3 loop and a portion of the RFSJ2 molecule. However, they were not definitive as we chose 30% of positive control as an arbitrary threshold of cross-reaction.

RFSJ2 and PAMLN6 inhibit the binding of anti-SIVMac251gp120 mMoAbs, KK17 and KK46, to SIVMac251gp120

As inhibition assays are known to be very sensitive [22], and because the epitope(s) shared between SIVMac251gp120 may be conformational and prone to be lost during adsorption onto the solid phase [23], we

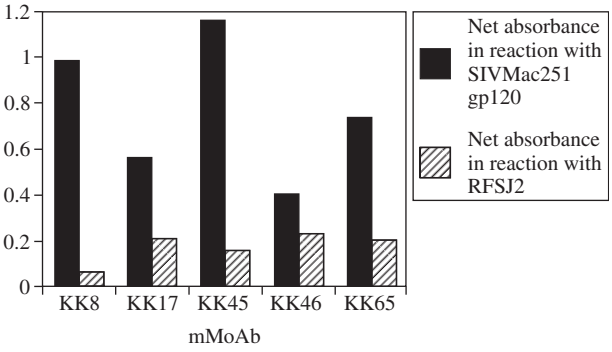

Figure 1 Results of screening of anti-SIVMac251gp120 murine monoclonal antibodies (mMoAbs) for their ability to cross-react with a human monoclonal immunoglobulin (HMIg), RFSJ2. Wells of polystyrene plates were coated with SIVMac251gp120 or RFSJ2, and indirect enzyme-linked immunosorbent assay (ELISA) was performed as described in *Materials and methods*. In the ELISA, a panel of mMoAbs of the KK series [16, 17] was used as primary antibodies. Shown are net absorbance values in reaction of the indicated KK mMoAb with SIVMac251gp120 (solid bars) or with RFSJ2 (shaded bars).

decided to verify whether HMIgs inhibit the binding of anti-SIVMac251gp120 mMoAb to the retroviral protein. We took advantage of the fact that the binding of the anti-SIVMac251gp120 mMoAbs of the KK series, which were available to us, to the SIVMac251gp120, was always strong and reproducible. The KK17 and KK46 antibodies were diluted so that their specific binding to the viral protein in the indirect ELISA (see *Materials and methods*) resulted in absorbance that equalled 0.5–1.0. Preliminary experiments showed that this dilution was 1 : 200 for both mMoAb preparations supplied by the NIHARRP (see *Materials and methods*). In the inhibition assay, samples of KK17 and KK46 at the dilution 1 : 100 were incubated with the increasing concentrations of HMIg, and the residual binding of the mMoAb to SIVMac251gp120 was tested as described in the *Materials and methods*.

The results of the inhibition assay are shown in Tables 2 and 3. As shown, four HMIgs, namely, RFKL1, PRTS2, PAMLN5 and AH-1 had no effect on the binding of KK17 to SIVMac251gp120. However, the binding of KK17 to SIVMac251gp120 was weakly (up to 34%), but significantly ($P < 0.05$), inhibited in the presence of RFSJ2 (Table 2). In addition, the binding of KK17 to SIVMac251gp120 was weakly (up to 39%), but significantly ($P < 0.05$), inhibited in the presence of another HMIg, PAMLN6 (Table 2). The binding of KK46 to SIVMac251gp120 was not affected by RFKL1, PRTS2, PAMLN5 and AH-1, but it was somewhat inhibited by PAMLN6 (up to 23%, $P < 0.05$) (Table 3). The binding of KK46 to SIVMac251gp120 was also very slightly (only up to 17%), but significantly ($P < 0.05$), inhibited in the presence of RFSJ2 (Table 3). The binding of both KK17 and KK46 to SIVMac251gp120 was profoundly (on average, up to 87%) and significantly ($P < 0.05$) inhibited by SIVMac251gp120 under the conditions of these experiments (not shown).

RFSJ2 and PAMLN6 do not react with murine IgG

The apparent inhibition of the binding of the mMoAbs KK17 and KK46 to SIVMac251gp120 in the presence of HMIg, RFSJ2 or PAMLN6, could be caused by the 'consumption' of the mMoAbs, because of the binding of the mMoAbs to an epitope on the HMIg, which was presumably similar to the epitope on the viral protein. However, an alternative explanation could be that RFSJ2 or PAMLN6 bound an epitope on the mMoAbs, thus preventing them from binding to SIVMac251gp120. To address this possibility, we tested the binding of RFSJ2 and PAMLN6 to murine IgG, or to hIgG (Calbiochem), in indirect ELISA. The results are shown in Fig. 2. As expected, RFSJ2 or PAMLN6 both vigorously bound hIgG. However, no binding of these HMIgs to solid-phase murine IgG was observed in the same experiment (Fig. 2). Thus, the observed inhibition of binding of KK17 and KK46 to SIVMac251gp120 in the presence of RFSJ2 and PAMLN6 seems to be more consistent with the mMoAb binding an epitope on the HMIg, rather than with the HMIg binding an epitope on the mMoAb.

Discussion

This paper describes experiments addressing the question: can antibodies elicited against REPs bind hIgs *in vitro*? The rationale for asking this question was that previously, structural similarities between REPs and hIgs were discovered [10–12]. Amino acid sequence homologies were found between some portions of HIV-1, HIV-2 and SIV envelope proteins and hIg V_H regions [13]. Can these structural homologies create epitopes shared by REPs and hIgs, especially their V regions? If so, one might expect that the binding of anti-REP antibodies to hIgs *in vivo* may occur, and lead to functional consequences that may be at least in

Table 2 Inhibition of the binding of KK17 to SIVMac251gp120 by different human monoclonal immunoglobulins

HMIg concentration*	RFSJ2 ($n=8$)	RFKL1 ($n=4$)	AH-1 ($n=6$)	PAMLN6 ($n=6$)	PAMLN5 ($n=7$)	PRTS2 ($n=3$)
I	18.4 ± 6.0†	−16.7 ± 16.2	9.9 ± 8.7	19.1 ± 6.1†	11.5 ± 19.6	−2.8 ± 13.3
II	16.3 ± 6.7†	11.8 ± 3.1†	17.9 ± 8.8	17.7 ± 6.9†	10.1 ± 19.9	−7.8 ± 12.6
III	14.6 ± 6.7	18.5 ± 8.1	10.9 ± 9.6	25.2 ± 6.1†	9.1 ± 19.6	8.2 ± 10.3
IV	21.6 ± 4.7†	5.1 ± 2.5	7.8 ± 10.2	20.0 ± 8.3†	45.0 ± 19.1	3.2 ± 5.5
V	21.9 ± 6.5†	21.8 ± 10.3	19.0 ± 8.0	18.4 ± 8.9†	39.0 ± 20.6	3.9 ± 5.3
VI	34.2 ± 6.9†	32.9 ± 11.5	19.9 ± 7.7	38.8 ± 4.8†	28.4 ± 16.6	11.8 ± 8.1

A fixed amount of KK17 was incubated with the indicated amounts of the HMIgs as detailed in *Materials and methods*, and then allowed to react with solid-phase-immobilized SIVMac251gp120. The binding without the HMIg was characterized by ~0.5–1 unit of net absorbance in enzyme-linked immunosorbent assay and was taken as 0% inhibition in each of the experiments. Shown are percentages of inhibition of the KK17 binding to SIVMac251gp120 in the presence of the HMIgs (means and standard errors of the mean of several independent experiments). HMIg, human monoclonal immunoglobulin; *n*, the number of independent experiments.

*Roman numerals stand for concentrations of the HMIgs in μg/ml. For RFSJ2, AH1, PAM LN5, PAM LN6 and PRTS2, they were as follows: I, 0.5; II, 2; III, 8; IV, 32; V, 100; VI, 200. For RFKL1, they were as follows: I, 0.5; II, 1; III, 3; IV, 6; V, 12; VI, 24.

†Asterisks indicate that the inhibition of the binding is significant ($P < 0.05$).

Table 3 Inhibition of the binding of KK46 to SIVMac251gp120 by different human monoclonal immunoglobulins

HMIg concentration	RFSJ2 ($n=8$)	RFKL1 ($n=4$)	AH-1 ($n=6$)	PAMLN6 ($n=6$)	PAMLN5 ($n=7$)	PRTS2 ($n=5$)
I*	−13.8 ± 7.3	10.2 ± 5.2	−8.2 ± 14.4	13.5 ± 1.0†	12.2 ± 8.9	8.5 ± 9.2
II	3.1 ± 6.4	7.9 ± 5.4	1.2 ± 6.8	13.7 ± 1.3†	14.6 ± 7.0	16.0 ± 9.5
III	10.9 ± 5.4	11.8 ± 3.4†	0.9 ± 6.8	17.7 ± 2.1†	14.6 ± 8.7	19.2 ± 9.9
IV	17.3 ± 3.6†	1.4 ± 5.4	0.5 ± 1.5	14.4 ± 1.6†	16.5 ± 6.9	15.9 ± 9.2
V	12.1 ± 6.0	−4.9 ± 7.4	15.1 ± 3.3	10.1 ± 2.7†	5.2 ± 8.6	15.3 ± 9.6
VI	14.4 ± 4.5†	13.3 ± 8.8	11.7 ± 5.4	23.5 ± 4.7†	10.8 ± 8.7	12.7 ± 13.5

A fixed amount of KK46 was incubated with the indicated amounts of the HMIgs as detailed in *Materials and methods*, and then allowed to react with solid-phase-immobilized SIVMac251gp120. The binding without the HMIg was characterized by ∼0.5–1 unit of net absorbance in ELISA and was taken as 0% inhibition in each of the experiments. Shown are percentages of inhibition of the KK46 binding to SIVMac251gp120 in the presence of the HMIgs (means and standard errors of the mean of several independent experiments). HMIg, human monoclonal immunoglobulins; *n*, the number of independent experiments.

*Roman numerals stand for concentrations of the HMIgs in μg/ml. For RFSJ2, AH1, PAM LN5, PAM LN6 and PRTS2, they were as follows: I, 0.5; II, 2; III, 8; IV, 32; V, 100; VI- 200. For RFKL1, they were as follows: I, 0.5; II, 1; III, 3; IV, 6; V, 12; VI, 24.

†Asterisks indicate that the inhibition of the binding is significant ($P<0.05$).

part responsible for the well-documented dysfunction of the humoral immunity in patients with retroviral infections.

In the present study, we concentrated on one commercially available REP, SIVMac251gp120 [17], and on a panel of mMoAbs that had been elicited against this protein, as well as some other SIV proteins in mice [16, 17]. The HMIg that we chose for this initial series of experiments was RFSJ2, previously shown to have an amino acid homology with some REPs [13]. We initially attempted to test whether these mMoAbs bind RFSJ2 in an indirect ELISA. None of the mMoAbs showed strong and consistent binding to RFSJ2 in this assay. However, two of the anti-SIVMac251gp120 mMoAbs did show a rather weak, but noticeable and consistent cross-reaction with RFSJ2, their binding being 40–50% of the positive control (Fig. 1). Notably, both of these mMoAbs are specific to the V3 loop of

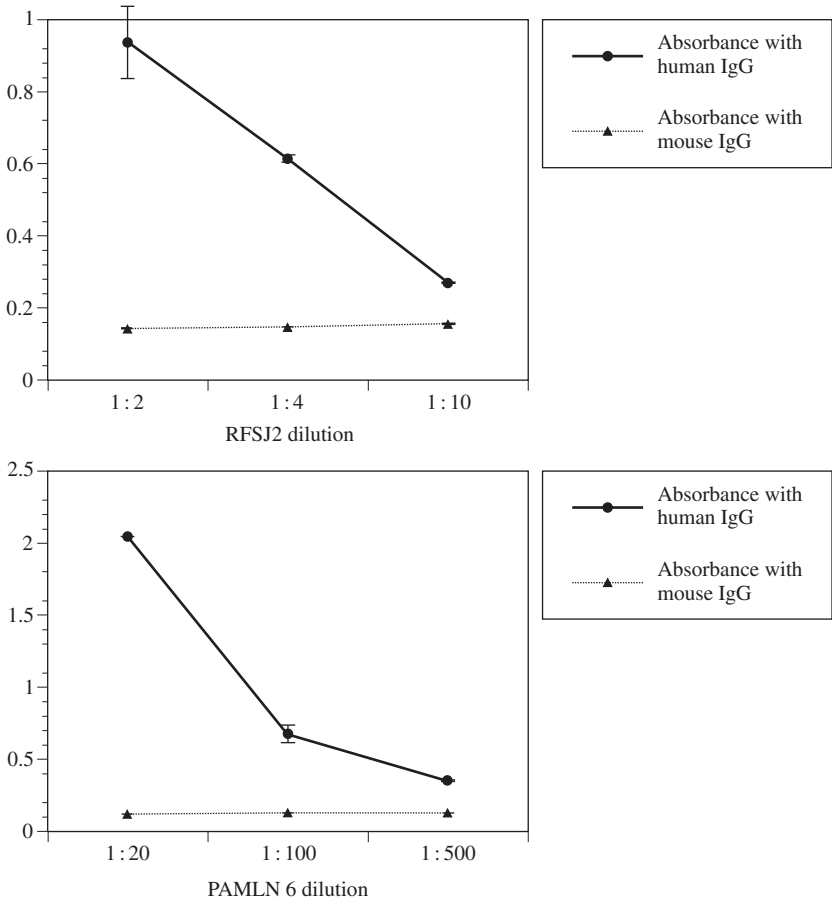

Figure 2 Indirect enzyme-linked immunosorbent assay showing no apparent binding of human monoclonal immunoglobulins (HMIgs) to murine IgG. RFSJ2 (upper panel) or PAMLN6 (lower panel) were allowed to react with solid-phase-adsorbed hIgG (straight lines) or with solid-phase-adsorbed murine IgG (dotted lines). See *Materials and methods* and *Results* for details. Shown are net absorbance values (means and standard errors of the mean of two experiments).

SIVMac251gp120 [16, 17], which is where the tetrapeptide NKYY, shared between RFSJ2 V_H region and some REPs, was found [13]. The two other anti-SIVMac251gp120 mMoAbs, KK42 and KK45, which bind an epitope located close to the one bound by KK46 (amino acid residues 311–340 [17]), showed a very weak cross-reaction that was considered below the 30% threshold (Fig. 1 and results not shown). Four other anti-SIVMac251gp120 mMoAbs, KK65, KK68, KK15 and KK41, that bind the viral protein outside of its V3 loop [17], and two anti-SIVMac251 mMoAbs that are specific to proteins other than gp120 (KK59 and KK64) showed no binding to RFSJ2 (results not depicted). Thus, although not definitive, these results suggested that epitope(s) shared between SIVMac251 envelope proteins and RFSJ2 may exist, likely to be located within the V3 loop of the SIVMac251gp120.

In indirect ELISAs, where the HMIg is adsorbed onto polystyrene plates, there is a possibility of epitope masking, giving rise to the rather weak reactions that were observed. To avoid this possible epitope loss on RFSJ2, we decided to employ an inhibition assay where the HMIg would compete with anti-SIVMac251gp120 mMoAbs for the binding to the adsorbed viral protein, the HMIg being in the liquid phase. In these experiments, the binding of KK17 and KK46 to its epitope on SIVMac251gp120 was weakly, but consistently, inhibited in the presence of RFSJ2 and also another HMIg, PAMLN6 (Tables 2 and 3). This inhibition seemed not likely to be attributed to a cross-reaction of RFSJ2 or PAMLN6 with murine IgG (which could affect the mMoAb antigen-binding site either directly or because of an allosteric effect), as neither RFSJ2 nor PAMLN6 showed binding to murine IgG in an indirect ELISA (Fig. 2). Thus, the inhibition experiments added some more confidence to the notion that KK17 and KK46 cross-reacted with the HMIg, resulting in the observed inhibition of these mMoAbs' binding to SIVMac251gp120, or, in other words, that RFSJ2 and PAMLN6 may contain epitope(s) similar to those recognized on SIVMac251gp120 by KK17 and KK46.

Our results, however, may not indicate that the epitope(s) bound by KK17 and KK46 on SIVMac251gp120 and on the HMIgs, RFSJ2 and PAMLN6, are structurally identical. The affinity of the binding of these mMoAbs to the HMIgs is, apparently, very low, because, as judged from the inhibition assay (Tables 2 and 3), the competition for the epitope(s) is weak even when very large (microgram) concentrations of HMIgs are present. This may mean that the epitope(s) on the HMIgs are somewhat modified compared with the epitopes KK17 and KK46 recognized on the SIVMac251gp120. One reason for that may be a point mutation, which is known to be able to decrease the affinity of an antibody binding dramatically [24]. Alternatively, as we carried out our inhibition assay at +4 °C, the observed decrease in the residual binding of KK17 and KK46 with SIVMac251gp120 after the preincubation with RFSJ2 and PAMLN6 may be explained by a cryoprecipitation of the Ig molecules [25]. Indeed, cryoprecipitation is characteristic for HMIgs that belong to IgM class and possess the activity of rheumatoid factors [25, 26]. It has been shown that some IgM antibodies can form cryoprecipitates with IgG via both Fc–Fc and Fc–Fv interactions [25] or by Fv–Fv interactions [26], albeit the IgG in these studies were of human and not of the murine origin like our anti-SIVMac251gp120 antibodies. Murine IgG can also form cryoprecipitates [27], but these antibodies are of the IgG3 subclass, while KK17 is an IgG2a and KK46 IgG1 [16, 17](Table 1). Besides, neither KK17 nor KK46 are rheumatoid factors, while the cryoprecipitating murine Igs were shown to be rheumatoid factors [27]. Thus, although it cannot be entirely ruled out that in our experiments, the residual binding of KK17 and KK46 to SIVMac251gp120 diminished after the preincubation with HMIgs, because the mMoAbs and/or the HMIgs formed cryoprecipitates, this explanation seems unlikely. Most importantly, cryoprecipitation does not explain why RFSJ2 and PAMLN6 cause the diminution of the subsequent mMoAb binding to the antigen and other HMIgs do not. Additional experiments where different regimes of the inhibition assay are studied in this regard are under way in our laboratory.

Both inhibiting and noninhibiting HMIgs are of the IgM class [18–20] (Table 1), which argues against association of the cross-reactive epitope with the constant region of the HMIgs' heavy chain. The location of this epitope on the constant region of the HMIGs' light chain is possible but also unlikely, because the lambda isotype was represented in both inhibiting (RFSJ2 and PAMLN6) and noninhibiting (AH1, PRTS2 and PAMLN5) HMIgs [18–20] (Table 1). Therefore, a more likely possibility is that the KK17 and the KK46 cross-reactive epitope(s) are located in the variable regions of the HMIgs. As two different V_H regions are utilized by the two HMIgs that inhibit KK17 and KK46 (V_H3–30.3 by RFSJ2 and V_H5–51 by PAMLN6 [18–20], and Table 1), it is plausible to suggest that the epitope(s) seen by KK17 and KK46 in RFSJ2 and PAMLN6 and cross-reactive with SIVMac251gp120 are different from the 7B4 idiotope [13]. They may be, rather, associated with, or located not in the V_H, but in the λ chain variable region (V_λ) of the hIg molecule. Further studies aimed at testing this possibility by elucidating the structure of the PAMLN5 and PAMLN6 V_λ regions are now under way in our laboratories. In particular, we intend to set up binding assays with synthetic peptides corresponding to the V_H3–30.3-coded 7B4 idiotope (NKYY) as well as to the idiotopes detected within the human V_λ1 product.

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