No evidence for cross-reactivity of virus-specific antibodies with HLA allo-antigens

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Abbreviations

BCA: Background corrected mean fluorescence intensity

BKV: BK polyomavirus

CDC: complement-dependent cytotoxicity

CMV: cytomegalovirus

EBV: Epstein-Barr virus

HIV: human immunodeficiency virus

HLA: human leucocyte antigen

mAb: monoclonal antibody

MFI: mean fluorescence intensity

SAB: single antigen bead

VZV: varicella zoster virus

Abstract

Background

Antibodies directed against human leucocyte antigens (HLA) can develop through pregnancy, blood transfusions or organ transplants. Anecdotal evidence suggests that virus-specific antibodies may have the capacity to cross-react with HLA, a phenomenon called heterologous immunity, which is well described for T cell alloreactivity.

Methods

To determine whether antibody cross-reactivity between viral antigens and HLA is common, we tested 51 virus-specific human monoclonal antibodies (mAbs) specific for human immunodeficiency virus (HIV), varicella zoster virus (VZV), cytomegalovirus (CMV), and parvovirus, for reactivity against HLA class I and class II in single antigen bead assays. In addition, we tested the reactivity of 41 HLA-specific human mAbs against common viral antigens of CMV, VZV, HIV, Epstein-Barr virus, and BK polyomavirus.

Results

No cross-reactivity of any of the virus-specific mAbs with either HLA class I or class II molecules, as well as no cross-reactivity of any of the HLA-specific mAbs with any of the viral antigens was observed.

Conclusions

These findings indicate that the frequency of cross-reactivity on the antibody level between viral antigens and HLA, if present at all, is low. The emergence of HLA antibodies upon viral infection or vaccination is therefore probably due to bystander activation of dormant HLA-specific memory B cells.

Introduction

It is well recognized that sensitization against human leucocyte antigens (HLA) can be caused by pregnancies, blood transfusions, and organ transplants. Sensitization leads to the formation of alloantibodies, but also to circulating alloreactive memory B cells and T cells. Counter-intuitively, relatively high frequencies of alloreactive memory T cells exist in individuals not having experienced any of the sensitizing events described above.[1] This is explained by a phenomenon called heterologous immunity, in which memory T cells specific for viral peptides presented in self-HLA can respond to foreign HLA.[2] This cross-reactivity is due to the structural similarity between the complex of viral peptide with self-HLA, and allogeneic HLA.[3] Cross-reactivity appears to be a common feature of virus-specific T cells, with HLA reactivity described for T cells formed against Epstein-Barr virus (EBV),[4,5] cytomegalovirus (CMV),[6,7] varicella zoster virus (VZV),[6-8] Influenza A,[6,7] and human immunodeficiency virus (HIV).[9] Cross-reactivity between virus-specific T cells and allogeneic HLA appears to be rather common, since it was shown that 80% of virus specific T cell lines and 45% of virus-specific T cell cross-reacted to allogeneic HLA.[6] Such cross-reactivity works both ways, as allo-HLA stimulated T cells can lyse both EBV and CMV peptide-loaded autologous cells.[10]

Whether similar cross-reactivity of virus-specific antibodies with foreign HLA exists remains elusive. Increased HLA antibody formation upon viral infection or vaccination has been described anecdotally,[11-13] although this could be due to either heterologous immunity, bystander activation of dormant B cell clones, or a combination of the two.[14] If viral infections would lead to *de novo* HLA antibody development, screening for HLA antibodies upon viral infections in patients awaiting an organ transplant may be required,[11] although this notion is highly controversial.[15,16] To determine whether cross-reactivity of antibodies between viral antigens and HLA is likely to exist, we tested 51 virus-specific human

monoclonal antibodies (mAbs) with specificity for HIV-1, VZV, CMV or parvovirus, against HLA class I and HLA class II molecules in single antigen bead (SAB) assays. In addition, we tested the reactivity of a panel of 41 HLA-specific human mAbs against various viral antigens. We hypothesized that a proportion of human virus-specific mAbs would react to HLA, and/or a proportion of human HLA-specific mAbs would react to viral antigens, in case heterologous immunity on the level of humoral immunity would be prevalent.

Materials and methods

Human monoclonal antibodies

Virus-specific human mAbs were obtained from various sources and were specific for different viruses (table 1). The HIV-1-specific mAbs 37G12, 2F5, 4E10, 3D6, 5F3, 4B3, B12, PG16, PG9 and 2G12 were purchased from Polymun Scientific (Klosterneuburg, Austria). The HIV-1 specific mAbs 697, 830A, 447-52-D, 1357, 1361, 1393A, 2158, 2297 and parvovirus B19-specific mAb 1418 were kindly provided by Dr. Miroslaw Gorny.[17] The following reagents were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 specific mAbs 3869, 3074, 2191, 2219, 2442, 257-D IV, 268-D IV, 71-31, 240-D, 50-69, 91-5, 98-6, 246-D, 126-7 from Dr. Susan Zolla-Pazner,[18-28] and HIV-1 specific mAbs CH01, CH02, CH03, CH04, VRC-CH31, CH38, CH57, CH58, CH59, CH90, HG107, HG120 from Drs. Barton F. Haynes and Hua-Xin Liao.[29-32] The VZV-specific mAb rec-RC IgG was a generous gift from Dr. Randall Cohrs,[33] and the CMV mAbs 10B7, 8C10, 1F7, 26A1 and 13D9 were kindly provided by Dr. Ada Funaro.[34]

HLA-specific human mAbs were produced by cloned B cell hybridomas derived from pregnancy immunized individuals.[35,36] All HLA-specific mAbs were selected on basis of lysis of HLA-bearing target cells in complement-dependent cytotoxicity (CDC) assays and were of the IgG isotype. The reactivity and HLA specificity of these mAbs have been

confirmed in SAB assays. An overview of the characteristics of the 41 HLA-specific mAbs tested for virus cross-reactivity can be found in table 2. HLA specific-mAbs were used as hybridoma supernatants in which IgG concentrations ranged from 1 to 399 µg/ml (median 20 µg/ml).

Virus-specific IgG antibody detection

All virus-specific IgG assays were performed at the Medical Microbiology department of the LUMC, following protocols used for standard patient care. For determining reactivity against VZV and EBV, chemiluminescence immunoassays (Liaison VZV IgG and Liaison EBV VCA IgG, Diasorin, Saluggia, Italy) were used following the manufacturer's instructions. Both assays were analyzed using a Liaison XL chemiluminescence analyzer (Diasorin). CMV and HIV reactivity was determined by chemiluminescent microparticle immunoassays (Architect CMV IgG and Architect HIV Ag/Ab Combo tests (Abbott, Hoofddorp, the Netherlands), following the manufacturer's instructions. These assays were analyzed using an Architect immunoassay analyzer (Abbott). Finally, BK polyomavirus (BKV) reactivity was assessed by in-house Luminex technology as described previously.[37]

HLA antibody detection

Virus-specific mAbs were tested for HLA reactivity using Lifecodes single antigen class I and class II kits (Immucor Transplant Diagnostics, Stanford, USA). All samples were diluted to a median concentration of 10 µg/ml (range: 0.9-14.5 µg/ml) prior to testing. Briefly, virus-specific mAbs were incubated with HLA class I and class II single antigen beads in filter plates (Millipore, Billerica, USA) for 30 min in dark at room temperature. Following a wash step, goat anti-human IgG antibody conjugated to phycoerythrin was added to all wells and incubated for 30 min. After a second wash step, samples were measured on a Luminex

platform (LabScan 100) to obtain fluorescence intensities. Data analysis was performed using MATCHIT! antibody software version 1.3.1 (Immucor Transplant Diagnostics).

Results

To test whether human antibodies generated against viral antigens react against HLA molecules, we obtained 51 virus-specific human mAbs and tested them in SAB assays, covering 30 HLA-A, 48 HLA-B, 18 HLA-C, 38 HLA-DR, 31 HLA-DQ and 27 HLA-DP antigens. Background corrected mean fluorescence intensity (BCA) values were below zero for all HLA coated beads in all samples (exemplified in figure 1).

To determine whether cross-reactivity with viral antigens could be detected for HLA-specific antibodies, we selected 41 HLA-specific human mAbs, covering a wide range of HLA epitopes recognized.[36,38,39] These mAbs were tested against common viral antigens of VZV, EBV, CMV, HIV, and BKV in routine assays that are in place for clinical samples in our hospital. As can be seen in Table 3, no cross-reactivity of any of the mAbs with any of the viral antigens was observed.

Discussion

Evidence for the presence of antibody cross-reactivity between viral antigens and HLA molecules is scarce and anecdotal. Increased levels of HLA antibodies upon viral infection or vaccination could be either due to bystander activation of (memory) B cell clones, or due to true heterologous immunity.[14] Thus far, no studies have been performed with the aim to distinguish between these two scenarios. Assaying polyclonal sera from HLA immunized patients is unlikely to provide a clear answer, since it is not possible to discriminate between true responses against viral antigens and cross-reactivity. The availability of virus-specific and HLA-specific human mAbs allowed us to determine whether virus-specific antibodies

can react with HLA antigens, and vice-versa, in a controlled manner. Making use of clinically validated assays used for patient diagnostics allowed for reliably analyzing the reactivity to common HLA alleles and viral antigens.

We analyzed the reactivity of 51 human virus-specific monoclonal antibodies against a total of 192 HLA alleles, resulting in 9792 possible combinations. If heterologous immunity of the antibody compartment would be a common feature, like heterologous T cell immunity, at least some, if not several positive reactions would have been expected. Concomitantly, we analyzed the reaction patterns of 41 human HLA-specific monoclonal antibodies with antigens of 5 different, some highly prevalent viruses, resulting in 205 possible combinations. This analysis again showed a complete lack of cross-reactivity, making the existence of heterologous immunity in the antibody compartment an event with very low prevalence, if present at all.

The use of a total of 92 monoclonal antibodies allowed for a straightforward analysis in a clean testing environment. These monoclonal antibodies represent high affinity B cell clones producing IgG antibodies, which leaves the possibility that heterologous immunity may be present in low affinity B cells, which we could not address in our assays. Our previously performed T cell analyses on virus-specific T cells clones showed that at least for T cells, high affinity virus-specific clones were cross-reactive.[6] While serum analysis possibly could be useful to address the point of low affinity B cell crossreactivity, the possible admixture of HLA specificities and virus specificities without knowing whether the immunizing event was HLA or virus, would make drawing solid conclusions very difficult.

Interestingly, for many neutralizing HIV-1-specific human monoclonal antibodies it has been described that they are polyreactive and can bind with high avidity to mammalian autoantigens.[40] Indeed, for at least two of the HIV-specific monoclonal antibodies described

herein (2F5 and 4E10), human kynureninase and splicing factor 3b subunit 3 have been identified as the primary autoantigenic targets.[41] Nonetheless, no reactivity towards HLA could be detected, even whit the highly sensitive SAB Luminex analysis. Additional testing of such polyreactive antibodies may provide useful information in the future.

The apparent contradiction between absence or low frequency of antibody crossreactivity and the relatively high frequency of T cell crossreactivity with allogeneic HLA (45% of all T cell clones are crossreactive) can be explained on theoretical grounds. T cells are educated in the thymus to recognize self HLA with a peptide, albeit with low to intermediate affinity.[14] This results in a relatively high chance to recognize other HLA antigens that are structurally highly homologous. In contrast, B cells are not selected on reactivity with self-HLA presenting peptide. Rather, all self-HLA reactive B cells will be eliminated in the bone marrow at the early developmental stage.

The data presented here suggest that the presence of crossreactivity in the B cell compartment at levels relevant to clinical transplantation is highly unlikely. The emergence of HLA antibodies upon viral infection or vaccination is therefore likely due to bystander activation of dormant HLA-specific memory B cells. In light of the many thousands of virus-specific monoclonal antibodies that have been generated, this study represents a first step in determining whether heterologous immunity exists on the humoral level. Furthermore, assays to determine the presence of HLA-specific memory B cells are available,[42-45] allowing to formally address the question whether HLA antibody formation upon viral infections or vaccination is due to bystander activation of dormant HLA-specific memory B cells.

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Figure legends

Figure 1. Virus-specific monoclonal antibodies show no cross-reactivity with HLA molecules. Panel A and B show representative examples of virus-specific antibody reactivity towards HLA in Luminex single antigen bead assays. Background corrected mean fluorescence intensity (BCM) values are shown, which were negative for all virus-specific monoclonal antibodies tested. Panel C shows the negative and positive control values for all Luminex single antigen bead assays performed. MFI: mean fluorescent intensity, nc: negative controls, pc: positive controls.

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Tables

Table 1. Virus-specific monoclonal antibodies tested.

Clone	Specificity	Isotype	Epitope
37G12	HIV-1 p24	IgG1, κ	
2F5	HIV-1 gp41	IgG1, κ	ELDKWA
4E10	HIV-1 gp41	IgG1, κ	NWFDIT
3D6	HIV-1 gp41	IgG1, κ	SGKLICTTA
5F3	HIV-1 gp41	IgG1, λ	QNQQEKNE
4B3	HIV-1 gp41	IgG1, λ	SGKLIC
B12	HIV-1 gp120 (CD4bs)	IgG1, κ	discontinuous
PG16	HIV-1 gp120 (V3/glycan)	IgG1, λ	discontinuous
PG9	HIV-1 gp120 (V3/glycan)	IgG1, λ	discontinuous
2G12	HIV-1 gp120 (carbo)	IgG1, κ	mannose?
447-52-D	HIV-1 gp120 (V3)	IgG3, λ	KRIHIGPGR
697	HIV-1 gp120 (V2)	IgG1, λ	discontinuous
830A	HIV-1 gp120 (V2)	IgG3, κ	discontinuous
1357	HIV-1 gp120 (V2)	IgG1, κ	discontinuous
1361	HIV-1 gp120 (V2)	IgG1, κ	discontinuous
1393A	HIV-1 gp120 (V2)	IgG1, κ	discontinuous
2158	HIV-1 gp120 (V2)	IgG1, κ	discontinuous
2297	HIV-1 gp120 (V2)	IgG1, λ	discontinuous
3869	HIV-1 gp120 (V3)	IgG1, λ	
3074	HIV-1 gp120 (V3)	IgG1, λ	
2191	HIV-1 gp120 (V3)	IgG1, λ	
2219	HIV-1 gp120 (V3)	IgG1, λ	
2442	HIV-1 gp120 (V3)	IgG1, λ	
257-D IV	HIV-1 gp120 (V3)	IgG1, λ	KRIHI
268-D IV	HIV-1 gp120 (V3)	IgG1, λ	HIGPGR
71-31	HIV-1 p24	IgG1, λ	
240-D	HIV-1 gp41	IgG1, κ	LLGIWGCSG
50-69	HIV-1 gp41	IgG1, κ	discontinuous
91-5	HIV-1 p24	IgG1, λ	
98-6	HIV-1 gp41	IgG1, κ	SLIEESQNQQEKNEQELLEL
246-D	HIV-1 gp41	IgG1, κ	QQLLGIWG
126-7	HIV-1 gp41	IgG1, λ	
CH01	HIV-1 V1V2	IgG1	
CH02	HIV-1 V1V2	IgG1	
CH03	HIV-1 V1V2	IgG1	
CH04	HIV-1 V1V2	IgG1	
VRC-CH31	HIV-1 (CD4bs)	IgG1	
CH38	HIV-1 (C1)	IgG1	
CH57	HIV-1 (C1)	IgG1	
CH58	HIV-1 (V2)	IgG1	
CH59	HIV-1 (V2)	IgG1	
CH90	HIV-1 (C1)	IgG1	
HG107	HIV-1 (V2)	IgG1	
HG120	HIV-1 (V2)	IgG1	
1418	Parvovirus B19 VP1	IgG1, κ	Not determined
Rec-RC IgG	VZV gH/gL	IgG1, κ	
10B7	CMV gB	IgG1	
8C10	CMV gB	IgG1	
1F7	CMV gH	IgG1	
26A1	CMV	IgG1	
13D9	CMV	IgG1	

Table 2. HLA-specific monoclonal antibodies tested.

Clone	**HLA-specificity defined by CDC**	**Isotype**
BOY6C1	B27/B73	IgG, κ
OK8F12	B72/B62/B46	IgG1, κ
HDG2G7	A32/A23/A25/B49/B38/B58	IgG1, κ
IND7D2	B21/B15/B52/B72/B56	IgG1, κ
DK1G8	A29	IgG1, κ
VTM1F11	B27/B7/B60	IgG1, κ
DK7C11	B12	IgG1, κ
VIE6C10	A23	IgG1, κ
WK1D12	B7/B27/B60	IgG1, κ
BVK1F9	B8	IgG1, κ
IND3H3	A9(weak)	IgG1, κ
WIM8E5	A1/A10/A11/A9/A29/A30/A31/A33/A28	IgG1, κ
WAR5D5	B7/B27/B42/B55	IgG1, κ
MUL4C8	A3/A11	IgG1, κ
SN607D8	A2/A28	IgG1, κ
HDG11G12	B62/B5/B35/B72 /B53	IgG1, κ
VTM4D9	B7/B27 weak	IgG1, κ
KLL5E10	B51/B52	IgG1, κ
VTM9A10	B7/B27	IgG1, κ
MUL9E11	B55/B57?	IgG1, κ
VIN1B10	A1/A11 (provisional)	IgG1, κ
GV2D5	A1	IgG1, κ
BOY2A7	A10, A11	IgG1, κ
RTLK1E2	DR3+DR5+DR6+DR8 (weak)	IgG1, κ
VR1H5	DR11 (weak)	IgG1, κ
RTLK10E12	DR11	IgG1, κ
VTM3A1	B7	IgG1, κ
HDG8D9	B51/B35	IgG1, λ
BRO11F6	A11	IgG1, λ
SN230G6	A2/B17	IgG1, λ
GV5D1	A1/A9 (not A*2403; A80 weak)	IgG1, λ
MUS4H4	Bw4/A24/A32/A25	IgG1, λ
OUW4F11	Bw6	IgG1, λ
DMS4G2	B70/B39/B50/B62/B45/B60/B61/B41 (provisional)	IgG1, λ
HDG4B1	B57/B63/A32/A25	IgG1, λ
MUL2C6	A3/A11/A24	IgG1, λ
KAL3D5	B51/B52/B77	IgG1, λ
SN7B12	B17	IgG1, λ
TL3B6	DPBB1*0101+0301+0901DP epitope 85 DEAV	IgG1, λ
ZEL4F11	B60/B61/B7/B55/B56/B18/B13/B8/B62/B35/B38/ B78/B81	IgG3, κ
ROU9A6	B12/B13/B40/B21/B41	IgG3, λ

Table 3. Results of HLA-specific monoclonal antibodies tested against common viral antigens of VZV, EBV, CMV, HIV, and BKV in routine clinical assays.

Clone	VZV IgG (mIU/ml. interpretation)		EBV VCA (IgG U/ml, interpretation)		CMV IgG (AU/ml, interpretation)		HIV IgG (S/CO, interpretation)		BKV (IgG MFI, interpretation)	
BOY6C1	<10,0	Negative	<10,0	Negative	0,0	Negative	0,13	Negative	69,5	Negative
OK8F12	<10,0	Negative	<10,0	Negative	0,0	Negative	0,11	Negative	65,5	Negative
HDG2G7	<10,0	Negative	<10,0	Negative	0,0	Negative	0,09	Negative	59,5	Negative
IND7D2	<10,0	Negative	<10,0	Negative	0,0	Negative	0,11	Negative	57,5	Negative
DK 1G8	<10,0	Negative	<10,0	Negative	0,0	Negative	0,15	Negative	60	Negative
VTM1F11	<10,0	Negative	<10,0	Negative	0,0	Negative	0,14	Negative	58,5	Negative
DK7C11	<10,0	Negative	<10,0	Negative	0,0	Negative	0,15	Negative	65	Negative
VIE6C10	<10,0	Negative	<10,0	Negative	0,0	Negative	0,12	Negative	61,5	Negative
WK1D12	<10,0	Negative	<10,0	Negative	0,0	Negative	0,24	Negative	61	Negative
BVK1F9	<10,0	Negative	<10,0	Negative	0,0	Negative	0,17	Negative	63,5	Negative
IND3H3	<10,0	Negative	<10,0	Negative	0,0	Negative	0,12	Negative	59,5	Negative
WIM8E5	<10,0	Negative	<10,0	Negative	0,0	Negative	0,13	Negative	59	Negative
WAR5D5	<10,0	Negative	<10,0	Negative	0,0	Negative	0,10	Negative	61	Negative
MUL4C8	<10,0	Negative	<10,0	Negative	0,0	Negative	0,11	Negative	56	Negative
SN607D8	<10,0	Negative	<10,0	Negative	0,0	Negative	0,18	Negative	59	Negative
HDG11G12	<10,0	Negative	<10,0	Negative	0,0	Negative	0,11	Negative	65	Negative
VTM4D9	<10,0	Negative	<10,0	Negative	0,0	Negative	0,13	Negative	63,5	Negative
KLL5E10	<10,0	Negative	<10,0	Negative	0,0	Negative	0,09	Negative	52	Negative
VTM9A10	<10,0	Negative	<10,0	Negative	0,0	Negative	0,08	Negative	57	Negative
MUL9E11	<10,0	Negative	<10,0	Negative	0,0	Negative	0,13	Negative	53,5	Negative
VIN1B10	<10,0	Negative	<10,0	Negative	0,0	Negative	0,12	Negative	56	Negative
GV2D5	<10,0	Negative	<10,0	Negative	0,0	Negative	0,11	Negative	56	Negative
BOY2A7	<10,0	Negative	<10,0	Negative	0,0	Negative	0,12	Negative	59	Negative
RTLK1E2	<10,0	Negative	<10,0	Negative	0,0	Negative	0,09	Negative	51	Negative
VR1H5	<10,0	Negative	<10,0	Negative	0,0	Negative	0,08	Negative	64	Negative
RTLK10E12	<10,0	Negative	<10,0	Negative	0,0	Negative	0,06	Negative	61	Negative
VTM3A1	<10,0	Negative	<10,0	Negative	0,0	Negative	0,09	Negative	63	Negative
HDG8D9	<10,0	Negative	<10,0	Negative	0,0	Negative	0,14	Negative	61,5	Negative
BRO11F6	<10,0	Negative	<10,0	Negative	0,0	Negative	0,10	Negative	61	Negative
SN230G6	<10,0	Negative	<10,0	Negative	0,0	Negative	0,12	Negative	79	Negative
GV5D1	<10,0	Negative	<10,0	Negative	0,0	Negative	0,14	Negative	60	Negative
MUS4H4	<10,0	Negative	<10,0	Negative	0,0	Negative	0,07	Negative	61,5	Negative

OUW4F11	<10,0	Negative	<10,0	Negative	0,0	Negative	0,20	Negative	54	Negative
DMS4G2	<10,0	Negative	<10,0	Negative	0,0	Negative	0,11	Negative	50,5	Negative
HDG4B1	<10,0	Negative	<10,0	Negative	0,0	Negative	0,10	Negative	49	Negative
MUL2C6	<10,0	Negative	<10,0	Negative	0,0	Negative	0,09	Negative	50,5	Negative
KAL3D5	<10,0	Negative	<10,0	Negative	0,0	Negative	0,07	Negative	60,5	Negative
SN7B12	<10,0	Negative	<10,0	Negative	0,0	Negative	0,09	Negative	61,5	Negative
TL3B6	<10,0	Negative	<10,0	Negative	0,0	Negative	0,07	Negative	58	Negative
ZEL4F11	<10,0	Negative	<10,0	Negative	0,0	Negative	0,11	Negative	54	Negative
ROU9A6	<10,0	Negative	<10,0	Negative	0,0	Negative	0,12	Negative	52	Negative

VZV: varicella zoster virus
EBV: Epstein-Barr virus
CMV: cytomegalovirus
HIV: human immunodeficiency virus
BKV: BK polyomavirus
MFI: mean fluorescence intensity
Cut-off MFI value for BKV: 478