# Human monoclonal HLA antibodies reveal interspecies crossreactive swine MHC class I epitopes relevant for xenotransplantation

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## ARTICLE INFO

## ABSTRACT

Crossreactivity of anti-HLA antibodies with SLA alleles may limit the use of pig xenografts in some highly sensitized patients. An understanding of the molecular basis for this crossreactivity may allow better selection of xenograft donors. We have tested 68 human monoclonal HLA class I antibodies (mAbs) for reactivity with pig lymphocytes from SLA defined pigs and found nine to be crossreactive. Eight of nine were broadly HLA reactive IgM-mAbs. The putative HLA epitopes for seven mAbs. were conserved in the aminoacid sequence of the SLA alleles studied. The lack of reactivity of a large number of mAbs largely correlated with the absence of the putative epitopes in the SLA alleles studied. We conclude that most patients with anti-HLA class I antibodies should be able to find pig donors lacking SLA antigens that cross react with their antibodies and that many of the crossreacting epitopes can be defined by analysis of shared epitopes in the aminoacid sequence of human and pig MHC antigens.

## 1. Introduction

With human organs in short supply, xenotransplantation is commonly considered a viable alternative to meet the increasing demand for graftable organs. Hyperacute rejection (HAR), due to preformed natural antibodies to the Gal $\alpha$1,3 Gal epitope (Gal), can largely be avoided by using genetically engineered pigs to reduce cell surface expression of this epitope (Yamada et al., 2005). Many potential transplant patients have great difficulty in finding a donor due to serum antibodies that react broadly with donor HLA antigens, which puts them at risk of HAR. Previous studies have shown that pig cell crossreactivity of HLA antibodies persists in immunized human sera after absorption of Gal antibodies (Taylor et al., 1998; Naziruddin et al., 1998), but correlation with specificities defined by panel reactive antibody (PRA) testing has been disputed (Bartholomew et al., 1997). Better definition of antibody

detectable crossreactivity between HLA and SLA antigens would therefore improve selection of potential pig donors for HLA sensitized patients. In this study, 68 human monoclonal antibodies (mAbs) to HLA class I antigens, generated in our laboratory, were tested for crossreactivity to SLA antigens. The HLA specificity patterns of these human mAbs range from narrow (reactive with private epitopes) to extremely broad (reactive with shared epitopes), similar to patterns found in allosensitized patients. The use of mAbs instead of allo-antisera avoids any need to remove anti-Gal antibodies. These mAbs were tested for complement dependent cytotoxicity (CDC) (Renard et al., 1988) to lymphocytes from seven lines of SLA inbred pigs, which have been characterized at the DNA level for each SLA allele (Smith et al., 2005a). These mAb reactivities, together with the definition of HLA epitopes, enabled identification within the pig MHC sequences of reactive epitopes that can be used in selecting xenograft donors.

## 2. Materials and methods

### 2.1. Human mAbs

Human monoclonal HLA antibodies were used as supernatants of heterohybridomas, which were derived by EBV transformation, fusion and cloning of B-lymphocytes from multiparous women. Selection of HLA antibody producing clones was based on CDC

reactivity in the supernatants. HLA types of the multiparous women, their mates and offspring were determined by serogical or molecular typing methods. The Medical Ethics Committee of Leiden University Medical Center approved the use of human donors for this study. The HLA-specificities of human mAbs (supplementary Table 1) were determined by CDC using twelve panels of serologically HLA typed human peripheral blood cells (panel sizes ranging from 80 to 270 cells, median size 262). Each human mAb was tested against 1–3 panels. The cell panels contained all serogically defined HLA antigens, except the rare alloantigens HLA-B46, -B59, -B73, -B75, -B76, -B77, -B78. The infrequent alloantigens HLA-B54 and -B76 were present on one and two human cells respectively, hence did not show up in the specificity patterns of any human mAbs.

## 2.2. Mouse mAb

mAb FK5, (recognizing a pan human leukocyte marker, also reactive with pig cells) was produced locally by Dr. Frits Koning (LUMC).

## 2.3. Cells

Pig mononuclear cells were isolated by Ficoll Hypaque sedimentation from heparinized blood. Cells were used from pigs of herds maintained at the Sinclair Research Center, Columbia, MO (Yucatan Miniature pigs containing the Hp-5.0(w), Hp-4b.0(x), Hp-6.0(y), Hp-7.0(z) haplotypes (previous nomenclature, in parentheses, is used throughout this paper for brevity); and at MGH (containing the Hp-2.0(a), Hp-3.0(c), Hp-4a.0(d) haplotypes). Each cell contained a unique combination of haplotypes, except for cells, typed as Hp-4b.0, and Hp-6.0, which were available from two pigs each.

## 2.4. CDC

CDC was carried out as described (Bruning et al., 1982) with mAb FK5 as positive control, and percentages cytotoxicity were converted to CDC scores (1–8 as recommended by ASHI).

## 2.5. Flow cytometry (FCM)

Human mAbs were incubated with $1.5 \times 10^5$ pig lymphocytes in 50 µl for 1 h on ice, washed, incubated with FITC conjugated rabbit F(ab′)$_2$ fragments of anti-human IgM or IgG (Dako, Glostrup, Denmark), washed, fixed with paraformaldehyde (1%), and analyzed on a FACSCalibur (BD Biosciences, San Jose, CA) equipped with CellQuest.

## 2.6. MHC sequence analysis

Pig MHC class I aminoacid sequences of 81 alleles were downloaded from ftp://ftp.ebi.ac.uk/pub/databases/ipd/mhc/sla/sla.123.prot.fasta, and incorporated in locally developed software. Insertions and deletions were manually accommodated to maximize alignment with HLA class I sequences.

## 3. Results

### 3.1. Scope of human HLA mAb reactivity

A total of 68 human mAbs were included in this study, encompassing 24 HLA-A locus, 35 HLA-B locus, 6 HLA-A/B locus and 3 HLA-C locus reactive mAbs (supplementary material, Table 1). EBV transformation favors the selection of IgM clones, however, both IgM ($n = 48$) and IgG ($n = 20$) isotypes were represented. All human mAbs in this study have unique HLA-specificity with the following exceptions; three mAbs specific for HLA-A2/A28, two mAbs each for HLA-A2/B17, HLA-A3, HLA-B7/B27, HLA-B7/B27/B60, HLA-B8, HLA-B12, HLA-B17 and two mAbs recognizing the same HLA-A shared determinant (OK4F10 and OK4F9). Some HLA antigens are recognized by multiple human mAbs, each recognizing unique epitopes as exemplified by HLA-B62, -B49 and -B72, which are reactive with 12, 11, and 11 human mAbs respectively (supplementary Table 1). In contrast, HLA-B42, -B55 and -B48 are recognized by only one human mAb each. The paucity of HLA-C directed human mAbs precludes full coverage of HLA-C antigens. In contrast, all HLA-A antigens and most of the common HLA-B antigens are recognized by at least one human mAb.

### 3.2. Reactivity of human HLA mAbs with haplotyped pigs

CDC testing returned nine mAbs as reactive on haplotyped pigs' cells (Fig. 1). The HLA reactivities of these mAbs are described in Table 1. Two mAbs of identical HLA specificity (OK4F9 and OK4F10) showed reactivity on all haplotyped pigs tested (Fig. 1). In contrast, seven mAbs react in polymorphic fashion on this panel of pig cells by CDC, and these specificities can therefore be attributed to reactions with the products of defined alleles. All mAbs returned clearly positive or negative CDC scores, except mAb OK5A3, for which the SLA specificity is difficult to assign due to two scores of magnitude 4 in its reactivity pattern (Fig. 1).

Antigen binding to antibody, and complement activation are two independent processes, which may not occur in coordinated fashion. We therefore performed FCM on haplotyped pig lymphocytes with the CDC reactive mAbs only, as lack of sufficient cell quantities precluded FCM analysis with the full panel of 68 mAbs. The FCM results are summarized in Fig. 2. The FCM reactivity of three mAbs, OK1C9, OK4F9 and OK4F10 was fully concordant with CDC results. The other mAbs showed incomplete concordance between the two test methods. The intermediate (score 4) CDC reactivity of mAb OK5A3 on cells with haplotype xz and dd is reflected in the absence of FCM signals on these cells, and the CDC scores of magnitude 6 on xx and cc haplotyped cells are duplicated with low intensity FCM signals. The mAb WK4E3 needs a double dose of x encoded antigens to achieve a FCM detectable signal and otherwise disagrees with its CDC reactivity by borderline reactivities of yy, cc and aa haplotypes. The mAb WK3D10 harbors a borderline positive FCM signal on the d haplotype besides a good signal on the w haplotype. Complete failure to bind in FCM, coinciding with ability to activate complement was seen for mAbs VDK8F7 and DMS1B10, which were, by CDC, specific for the x and the z haplotypes respectively. The mAb DK7C11, which is of IgG isotype and is poor in complement activation on HLA-B44 and -B45, shows strong FCM reactivity on SLA-aa haplotyped cells and weak yy reactivity, on top of the CDC reactive z haplotyped cells. With one exception, the "extra" FCM specificities were of weak staining intensity, and otherwise the FCM data corroborate the CDC derived mAb specificities. CDC positive/FCM negative specificities, occurring five times in four mAbs, have been observed on human cells also (Zoet et al., 2005) and are due to low mAb affinity, resulting in sensitivity to washing. There was significant correlation between the two test methods (chi square = 23.087; $p < 0.000001$; correlation coefficient 0.6509).

### 3.3. Identification of human HLA mAb reactive epitopes in SLA haplotypes
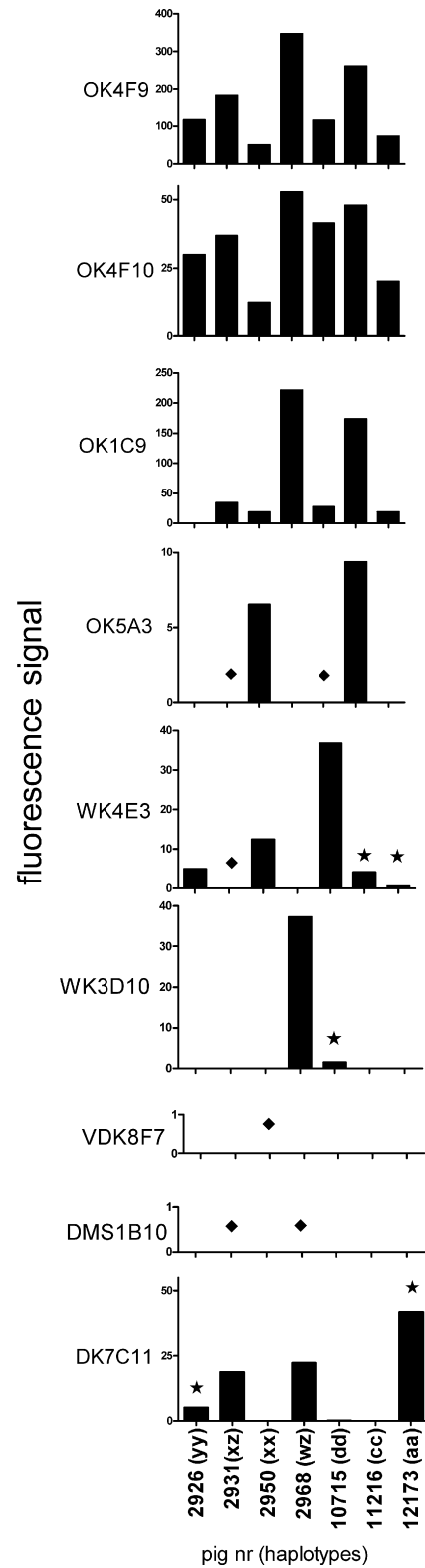
Conservation of epitopes across species barriers would explain the human-to-porcine crossreactivities of human HLA mAbs. Therefore, using the aminoacid sequences of the SLA alleles contained in these haplotyped pigs, we attempted to locate the human epitopes within the pig sequences, and correlate CDC defined pig haplotype reactivity. To guide this correlation Table 2 is included,
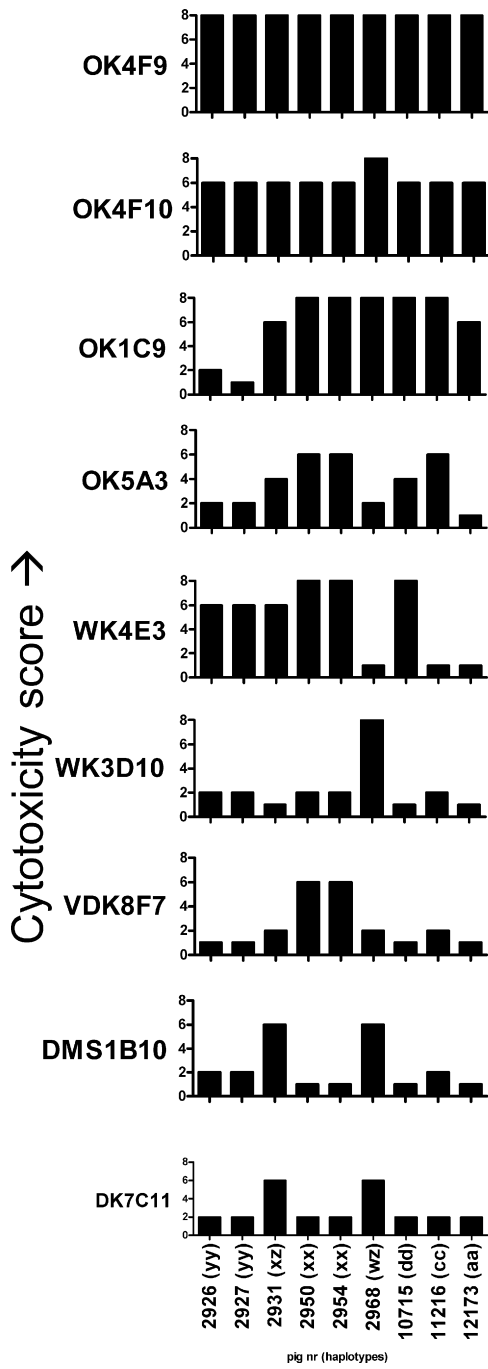
**Fig. 1.** CDC of human mAbs on haplotyped pig cells. Data of human mAbs showing at least one positive reaction are given, all other 59 human mAbs were negative; scores 1 and 2 considered negative. Cytotoxicity caused by mAb FK5 was 100%.



**Fig. 2.** Flowcytometry of human mAbs on haplotyped pig cells. Bars denote the fluorescence signal that was calculated as median channel of human mAb reactivity minus twice the background generated by anti-IgM or anti-IgG conjugate only. Note differences in scale. Discrepancies with CDC reactivities are marked: ♦ (CDC positive/FCM negative); and * (CDC negative/FCM positive).

**Table 1**
HLA specificity of pig cell reactive (by CDC) human mAbs.

| HuMAb | HLA reactivity by CDC | Isotype |
|---|---|---|
| OK4F9 | A1/A36/A3/A11/A34/A66/A26/A29/A30/A31/A33 | IgM |
| OK4F10 | A1/A36/A3/A11/A34/A66/A26/A29/A30/A31/A33 | IgM |
| OK1C9 | A3/A11/A33/A31/A26 | IgM |
| OK5A3 | A1/A3/A11/A24/A36 | IgM |
| WK4E3 | A locus (not A1, A23, A24) | IgM |
| WK3D10 | A2/A3/A23/A31/B7/B17/B13/B40/B21/B62 | IgM |
| VDK8F7 | Bw4 (not B13, B5, B53, B63) | IgM |
| DMS1B10 | B62/B21/B37/B56/B72/B52/B45 | IgM |
| DK7C11 | B12 | IgG |

**Table 2**
Allele distribution[1] among SLA haplotypes.

| Haplotype | Locus | | | Human mAb reactivity by CDC | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | SLA-1 | SLA-3 | SLA2 | OK4F9 and OK4F10 | OK1C9 | OK5A3 | WK4E3 | WK3D10 | VDK8F7 | DMS1B10 | DK7C11 |
| a | *0201 | null | *0201 | + | + | − | − | − | − | − | − |
| c | null | *0301 | *0301 | + | + | + | − | − | − | − | − |
| d | *0401 | *0401 | *0401 | + | + | + | + | − | − | − | − |
| x | *0401 | *0401 | *04sx01 | + | + | + | + | − | + | − | − |
| w | *0401 | *05sw01 | *08sw01 | +[2] | +[3] | − | − | + | − | +[4] | +[5] |
| y | *w08sy01 | *0601 | *05sy01 | + | − | − | + | − | − | − | − |
| z | *w08sz01 | *0701 | *05sz01 | +[2] | +[3] | + | − | − | − | + | + |

1. Extracted from Smith et al. (2005b).
2. Alternative explanations for OK4F9 and OK4F10 are reactivity with haplotypes x and z; or x and w; besides reactivity with y, a, c and d.
3. Alternative explanations for OK1C9 are reactivity with haplotypes x and z; or x and w; besides reactivity with a, c and d.
4. Alternative explanation for DMS1B10 is reactivity with the z haplotype only.
5. Alternative explanation for DK7C11 is reactivity with the z haplotype only.

**Table 3**
HLA characteristics of individuals used for hybridoma establishment.

| Individual | mAbs | Responder HLA class I phenotype | Immunizing HLA class I antigens[a] |
|---|---|---|---|
| OK | OK4F9 | A2, A28, B7, B27, Cw2, Cw7 | *A3*, B72 |
| | OK4F10 | | |
| | OK5A3 | | |
| | OK1C9 | | |
| WK[2] | WK4E3 | A1,-, B8,-, Cw7,- | A11, *A2*, B27, B65, Cw1, Cw8 |
| | WK3D10 | | |
| VDK | VDK8F7 | A3, A31, B35 (Bw6), Cw4, - | A1, *B37(Bw4)*, Cw6 |
| DK | DK7C11 | A1, A2, B8, B27, Cw2, Cw7 | A29, *B45*, Cw6 |
| DMS[b] | DMS1B10 | A3, A68, B51, B7, Cw7, - | A2, A29, *B62*, B35, Cw4 |

[a] Deduced from HLA typing of offspring. HLA antigens involved in immunization giving rise to hybridomas are italicised.
[b] Hybridomas WK4E3, WK3D10 and DMS1B10 were established from cells taken after second pregnancies.

which details the alleles contained in the seven haplotypes tested (see Smith et al., 2005b, Table 7 therein). The CDC reactivity (of Fig. 1) was entered to facilitate pinpointing mAb reactivities to allelic products.

The HLA epitopes responsible for binding human mAbs have been determined by the application of a modification of HLA-Matchmaker (Duquesnoy, 2002). This software, built for determination of antigenic segments in the human sequences, returns antigenic aminoacid triplets in antibody accessible sites of the HLA class I molecule, when HLA class I types of transplant patient/donor combinations and antibody reactive HLA antigens are entered by the user. Table 3 shows the HLA class I types of the women, from whom the pig reactive HLA mAbs were generated, as well as paternally derived antigens on offspring. These HLA typings, together with mAb specificities, were entered into HLA-Matchmaker. The human epitopes thus determined for mAbs are given in Table 4. To accommodate SLA class I sequences, besides HLA class I sequences, an adaptation of HLA-Matchmaker that returns single aminoacids, rather than triplets, was built locally. We used the CDC defined SLA specificities of the mAbs and searched for human epitopes in mAb reactive SLA alleles. This analysis, and the segregation of the epitopes among SLA alleles (Table 4) of nine mAbs are as follows.

We previously described (Duquesnoy et al., 2005) the epitope of mAbs OK4F9 and OK4F10 as the combination of 138M and 142I, with critical contributions from aminoacids in distant loops. Each pig haplotype has one allele containing the 138M/142I defined epitope, and the additional requirements for HLA class I binding (79GTLRG, position 76 not E) are fulfilled in these SLA alleles (81LRG, 76V). This invalidates the alternative SLA specificities offered in Table 2 (footnote 2).

The epitope recognized by mAb OK1C9 is a structural one, as it was undefinable based on HLA sequence analysis.

The HLA epitope of mAb OK5A3 was previously defined as 142ITKR, with critical contributions that are defined by the lack of reactivity of HLA-A80. The 142ITKR sequence is present in 10 of 16 alleles contained in the seven pig haplotypes studied here, but many of these allelic products are mAb OK5A3 nonreactive. The reactivity could be either due to epitopes shared by alleles

**Table 4**
Identification of human mAb reactive HLA epitopes in SLA alleles.

| mAb | Human epitope | SLA alleles containing human epitope[1] (haplotypes carrying it) | CDC reactive SLA haplotype(s) | Concordance by CDC |
|---|---|---|---|---|
| OK4F9 and OK4F10 | 138M 142I with 79GTLRG, if not 76E | SLA-1*0201(a), SLA-1*0401(dwx), SLA-1*08sy01(y), SLA-1*08sz01(z), SLA-2*0301(c) with 81LRG and 76V | a, c, d, x, w, y, z | Full |
| OK1C9 | Not defined | N/a | a, c, d, x, z | N/a |
| OK5A3 | 142ITKR | *SLA-1*0401(dwx)*, SLA-3*0401(dx), *SLA-3*05sw01(w)*, *SLA-3*0601(y)*, *SLA-3*0701(z)*, SLA-2*0401(d), SLA-2*04sx01(x), *SLA-2*08sw01(w)*, *SLA-2*05sy01(y)*, *SLA-2*05sz01(z)* | c, d, x | No |
| WK4E3 | 67V, if not 65G | SLA-1*0401(dwx), SLA-2*05sy01(y), *SLA-1*0201(a)* | d, x, y | Partial (SLA-1*0201 not reactive, SLA-1*0401 in w haplotype not reactive) |
| WK3D10 | Not defined | n/a | w | n/a |
| VDK8F7 | 82LR | SLA-2*04sx01 (x), *SLA-2*0401 (d)*, *SLA-2*05sy01 (y)*, *SLA-2*05sz01(z)* (82L only) | x | full if 95L |
| DMS1B10 | 65QIS | SLA-1*08sz01(z), *SLA-2*0201(a)*, SLA-2*08sw01(w), SLA-3*05sw01(w) | z, w | Partial |
| DK7C11 | 167S | SLA-2*05sz01 (z), *SLA-1*0401(dwx)* | z | Partial |

1. Allelic products that are not mAb reactive are italicised.

SLA-3*0301 and SLA-3*0401, or shared by SLA-2*0301, SLA-2*0401 and SLA-2*04sx01, not all of which harbour the 142ITKR epitope: the c haplotype alleles SLA-2*0301 and SLA-3*0301 both have a single aminoacid substitution at position 143 (S and A respectively). While mAb OK5A3 is obviously permissive to this substitution we were unable to pinpoint the nonreactivity with many other allelic products to other substitutions elsewhere in the molecule, such as those suggested by the nonreactivity of HLA-A80 (requiring either 74D or 151H for mAb reactivity).

For mAb WK4E3, the HLA epitope is 67V, but with a critical contribution from the aminoacid at position 65, because the presence of 65G is deleterious to the epitope, as exemplified by nonreactivity of HLA-A23 and HLA-A24. This is confirmed in CDC reactivity by pig cells haplotyped as x, y, and d but 67V is also present in the allele SLA1*0201 contained in the a haplotype, that is not reactive with the mAb WK4E3. In FCM, the a haplotype is reactive, but the c haplotype is also reactive, while neither of the c haplotype alleles, SLA2*0301 or SLA3*0301, has 67V (but they have Q or A respectively, neither of which occurs in any human allele at position 67).

The HLA epitope for mAb WK3D10 was undefinable based on HLA sequences alone. Ignoring the human sequences, we examined the w haplotype alleles SLA-3*05sw01 and SLA-2*08sw01 for unique aminoacid substitutions. The latter allele contains two unique substitutions, 45K and 72EI, and neither is present in any human allele. Further work using alleles with these substitutions should confirm this epitope.

The HLA epitope of mAbVDK8F7 (a short anti-HLA-Bw4 mAb, induced by immunization with HLA-B37) was defined by two aminoacids: 82LR. The requirement for 83R in this epitope is apparently not absolute, as the only SLA reactive allele, SLA-2*04sx01, requires just 82L for mAb reactivity. As the 82L is shared by three other SLA alleles tested, the epitope is likely further shaped by a critical contribution from another residue, 95L, which is the only unique substitution in close proximity, however not located in the α helix.

The mAb DMS1B10 reacts with a combination of allelic products, some of which have in common an epitope, 65QIS, present on the immunizing antigen HLA-B62, but the absence of the epitope on some mAb reactive HLA alleles precludes a definitive assignment of this epitope. The 65QIS epitope is present in four SLA alleles tested (two alleles being present in one cell), with three showing mAb reactivity. The added knowledge of SLA reactivity does not enable definitive epitope assignment for this mAb.

The mAb DK7C11 owes its HLA-B12 reactivity to a unique substitution, 167S, on HLA-B*4402, -B*4403 and -B*4501. The same substitution was present in the z haplotype allele SLA-2*05sz01, but also in the SLA-1*0401 that the d, w and x haplotypes have in common. Apparently, contribution of aminoacid(s) in close proximity, that distinguishe(s) these alleles (either or both 155GE and 169QK in SLA-2*05sz01) is essential for shaping this epitope. Although the CDC reaction pattern does not rule out the alternative SLA specificity of DK7C11 (for haplotypes z as well as w), it is invalidated with this analysis.

Thus, epitope sharing between human and pig alleles explained pig cell reactivity of two mAbs with full concordance and of three other mAbs with partial concordance.

### 3.4. Absence in pigs of immunogenic epitopes defined in HLA antigens

It was obvious from the CDC results that many human mAbs that detect immunogenic HLA epitopes failed to react with any of the pigs' cells. Given the good CDC signals of nine HLA mAbs on haplotyped pigs' cells as well as their confirmation by FCM, we argued that the pig cell nonreactivity of the remaining mAbs was not due to

low affinity, but due to the absence of reactive epitopes in pig alleles. Besides those serologically tested in the present study, many more SLA class I alleles have been characterized by sequencing. To get an up-to-date view of possible serological incompatibilities, we analyzed the aminoacid sequences of 81 pig MHC class I alleles for the presence/absence of serological HLA epitopes detectable by human HLA mAbs. We found nine human epitopes among the SLA alleles, which nevertheless were nonreactive with their counterpart eleven mAbs (supplementary Table 2). The presence of an HLA epitope may not be enough for antibody binding and mAb reactivity may require critical contribution(s) of aminoacid substitution(s) in close proximity in the HLA class I molecule, usually in adjacent coils (Duquesnoy et al., 2005). The elucidation of SLA nonreactivity of HLA mAbs will not be different. Thus, the anti-HLA-A1 mAb GV2D5 sees 44K in HLA-A1, but needs a critical contribution from 43Q, which however is absent in any pig allele. In other instances, mAb reactivity, despite the presence of a human epitope, may be abrogated by pig specific aminoacid substitutions in close proximity, as exemplified by mAb VP5G3. Absence of SLA reactivity of these twelve HLA mAbs is accounted for in supplementary Table 2. In contrast, the clarification of the absence of pig cell reactivity of the remaining mAbs was straightforward: the 21 human HLA epitopes, recognizable by 29 mAbs, simply are absent from all pig alleles (supplementary Table 2). Furthermore, there are 18 mAbs for which no epitope has been assigned to date by HLA sequence analysis, and which were nonreactive with pig cells. Taken together, the frequently occurring, pregnancy induced, antibody specificities such as anti-HLA-A2, -A3, -A11, -B7 and -B8 have no counterpart antigens in any pig alleles.

## 4. Discussion

The success of future xenotransplantation relies heavily on the occurrence of a good proportion of cross-match negative recipient–pig organ combinations. Highly immunized patients increasingly populate transplantation waiting lists (De Meester et al., 2002) and will be considered as primary candidates for clinical trials with xenotransplants. Patients' sera containing broadly reactive HLA antibodies (after Gal absorption and causing a high PRA and of IgG isotype) have been shown to be prone to crossreactivity with pig lymphocytes in one study (Oostingh et al., 2002), however, in another study (Wong et al., 2006), the proportion of crossreactive IgG antibodies within a set of similar sera, tested on lymphocytes from α1,3-galactosyltransferase gene knockout pigs, was low. Our study is the first to provide the molecular basis for the correlation between HLA class I antibody specificity and haplotyped pig MHC. Once SLA types of animals bred for xenotransplants will be available, the present data will be instrumental in predicting cross-match negative recipient-pig organ combinations.

In the present study, CDC was used to detect human HLA mAb reactivity with haplotyped pig lymphocytes, and subsequently FCM was used with the CDC identified pig reactive mAbs. We used CDC, not FCM, data for comparing human epitopes with pig sequences for the purpose of simplification, but also in view of the HAR mechanism, which is likely complement mediated. Although some human mAb reactivity may have gone undetected due to inability to activate complement in some combinations, the failure to activate complement may be an advantage for patients receiving pig organs. However two other mechanisms may have caused lack of cross-species mAb reactivity: SLA-3 locus alleles are expressed at lower density than SLA-1 and SLA-2 alleles (Vaiman et al., 1998; Sullivan et al., 1997; Frels et al., 1990) on pig cell surfaces, which provides an alternative explanation for lack of reactivity of two mAbs (BVK5C4 and VN2F1) (supplementary Table 2). Furthermore, an insufficient amount of relevant peptides in the SLA class I groove

may have prevented binding some of the mAbs (Mulder et al., 2005).

Out of nine mAbs reactive with haplotyped pig cells, for three (of which two were of identical specificity) the reactivity with human epitopes that were conserved in pigs was fully concordant, and for three other mAbs the CDC reactivities between pig and human were partially discordant, even though the epitopes were partly conserved. In the latter three mAbs, critical aminoacids outside the obvious epitope that preclude mAb binding were however not identifiable with the present data. The same goes for one mAb where concordance between epitope and haplotype reactivity was completely lacking. For the remaining two mAbs, the human epitope was not obvious from the human sequences to begin with, and the present addition of the pig data failed to facilitate identification of these conformational epitopes. Assuming similarity of human and pig class I 3D structures, all four mAb defined epitopes in SLA class I, as well as the tentative 65QIS epitope, are located in readily Ab accessible α-helices.

Here, it turns out that the majority (8 of 9) of mAbs, scoring CDC positive on pig cells, harbor broad HLA reactivity. As evident from earlier work (Duquesnoy et al., 2005) broad HLA reactivity can be due to a single antibody, reactive with an epitope shared with many alleles. It is intriguing that many of these broadly reactive antibodies are produced by individuals (3 of 5 antibody producers in this study, Table 3) who themselves are either homozygous at HLA class I loci or heterozygous, but containing alleles with a high degree of homology at one or more loci. There is logic in this: the likelihood that epitopes shared by many alleles are self-epitopes (and therefore not immunogenic) in heterozygous individuals is high. Even so, these individuals are able to mount antibody responses against private epitopes besides shared epitopes, as we have shown for the mAbs produced by individual OK (Duquesnoy et al., 2005). However, in routine serum analysis, the reactivity of these private Abs is obscured by the broadly reactive Abs.

The broadly reactive mAbs that were pig reactive in this study were all of IgM class, while the sole private mAb was of IgG class. This contrasts with the equal distribution of public and private reactivities among the isotypes in our mAb panel. IgM is considered closer to germline, and harbors less somatic mutation, than IgG. Apparently, somatic hypermutation that is concomitant with class switching to IgG due to secondary exposure to antigen (McHeyzer-Williams and McHeyzer-Williams, 2005) causes abrogation of xenoreactivity of the HLA alloresponse, as in just one (DK7C11) of four IgG mAbs with defined HLA epitopes the SLA reactivity is retained, despite maintenance of crossreactive epitopes across the two species (supplementary Table 2). The incidence of cross-species reactive HLA antibodies of IgM isotype in Gal absorbed sera has recently been reported (Diaz et al., 2003). Also in line with our findings is the recent demonstration that antibodies in patients' sera, when tested against Gal knockout pig cells (of dd haplotype), were predominantly of IgM isotype (Wong et al., 2006).

We showed here that HLA antibody reactivity can be pinpointed to a single allelic SLA protein product, or a limited number thereof. In the case that breeding animals for xenotransplantation will be limited to a few pig haplotypes, it would pay to develop serum tests, preferably for antibodies of both IgM and IgG isotypes, with single purified SLA antigens encoded by the individual alleles in these haplotypes. This obviates the need for laborious absorption of patients' sera, and will enable acceptable mismatching of pig organs to individual HLA (highly) immunized patients. A similar strategy has proven successful in human-to-human transplantation (Claas et al., 2004, 1999). Likewise, screening of a large cohort of patients' sera in such tests will identify SLA haplotype(s) with the minimum number of human antibody reactive epitopes. The next logical step would be the selection of Gal knockout MHC haplotyped pigs (Cooper et al., 2004) that contain a minimum number of epitopes that are recognizable by human antibodies.

In summary, the xenoreactivity of human HLA mAbs has permitted the identification of reactive epitopes that are conserved across the two species. To optimize xenograft survival, future xenotransplantation strategies should preferably include virtual recipient/donor matching for these epitopes that are predominantly recognized by broadly reactive HLA mAbs of IgM isotype.

## Acknowledgements

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molimm.2009.10.004.

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