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Research Article

Structural Differences in KIR3DL1 and LILRB1 Interaction with HLA-B and the Loading Peptide Polymorphisms: *In Silico* Evidences

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KIR3DL1 and LILRB1 interact with HLA class I. Using KIR3DL1/HLA-B interaction to set up the procedure, structural immune-informatics approaches have been performed in LILRB1/HLA-B alleles' combination also considering the contribution of the HLA bound peptide. All KIR3DL1 alleles interact strongly with HLA-B alleles carrying Bw4 epitope and negative charged amino acid residues in peptide position P8 disrupt KIR3DL1 binding. HLA-B alleles carrying Ile 194 show a higher strength of interaction with LILRB1 in all the analyzed haplotypes. Finally, we hypothesize a contribution of the amino acid at position 1 of the HLA bound peptide in the modulation of HLA-B/LILRB1 interaction.

1. Introduction

The modulation of NK cells' immune response is mediated by inhibitory and activating receptors expressed on their surface [1–4]. Among them, KIR3DL1 and LILRB1 have shown the capability to recognize HLA class I molecules.

KIR3DL1 is able to interact with HLA class I molecules carrying Bw4 epitope, while no interaction has been observed in the presence of Bw6 epitope. KIR3DL1 alleles are divided into three subclassifications depending on NK surface expression: high expressive (*001, *002, *008, *009, *015, and *020), low expressive (*005, *007), and nonexpressive (*004) [5, 6]. Association studies between KIR3DL1 alleles and infectious diseases have been extensively reported in the past years. In this context, high expressive and nonexpressive alleles lead to a delayed HIV infection progression in the presence of HLA-B carrying Bw4 epitope [7]. These results encouraged the evaluation of HLA/KIR3DL1 pattern of interaction [8–11]. Beside the strong interaction with HLA Bw4 epitope, structural evidences have also shown a role for the HLA bound peptide in the recognition by KIR3DL1 [12–17].

We have previously reported a system for analyzing the interaction between KIR3DL1*001 allele and different HLA-B alleles by using a docking approach recently confirmed by experimental data [14, 16, 18]. Due to the fact that interaction patterns in the context of different HLA/KIR3DL1 variants still need to be addressed the use of the same *in silico* approach could shed new light on the HLA/KIR3DL1 interaction that has not yet been evaluated.

LILRB1/ILT-2/LIR1 is an inhibitory receptor expressed on a fraction of NK cells, α/β and γ/δ T-cells, CD19+ B cells, CD14+ monocytes, and HLA-DR^{high} dendritic cells [19, 20].

In NK cells, the block of the LILRB1 receptor has shown the restoration of NK cell cytotoxic activity, suggesting a key role in the immune regulation [21].

LILRB1 is composed by four Ig-domains (D1–D4 form N to C terminal) and binds HLA-A, HLA-B, and HLA-G with high affinity [22–26]. LILRB1 interacts with HLA class I nonpolymorphic α3-domain [27] and β2-microglobulin domain [28, 29].

Amino acids Ala 193 and Val 194 were associated with lower binding property of LILRB1 even though apparently

Table 1: List of KIR3DL1 alleles selected for immune-informatics approach. Alleles were selected depending on amino acid polymorphisms with respect to the reference allele KIR3DL1*001 and allele frequency in general population [36, 37].

Expression	KIR3DL1 alleles	KIR3DL1 polymorphic amino acid position (7–292)					
		47	54	58	92	182	283
High	*KIR3DL1* 001*	I	I	S	V	P	W
	KIR3DL1 009*	**V**	I	**G**	**M**	P	W
	KIR3DL1 015*[#]	**V**	**L**	S	V	P	W
Low	*KIR3DL1* 007*[#]	**V**	**L**	S	V	P	W
	KIR3DL1 005*	I	I	S	V	**S**	**L**

[#]007 and 015 share the same amino acid polymorphisms and have been analyzed together.

the only presence of Val 194 did not influence the LILRB1 recognition [30]. We recently reported a direct association of Val 194 in HIV delayed progression due to a weaker interaction with LILRB1 receptor [31]. Thus, HLA amino acid position 194 could have a role in the modulation of LILRB1 interaction.

Twelve LILRB1 polymorphisms located between -634A>G to c.464G>T constitute three major haplotypes in Japan (LIR1.PE01, LIR1.PE02, and LIR1.PE03). Previous studies have associated LILRB1 haplotypes with viral disease progression and variation of NK cells' membrane expression [32–34]. In addition, the HLA bound peptide has shown to contribute in the interaction [35], suggesting a role in the modulation of the strength of interaction with LILRB1 similar to the one observed in KIR3DL1. However, peptide position and amino acid variants able to modulate the LILRB1 binding have not yet been assessed. The aim of this study is to evaluate the interaction pattern of KIR3DL1 and LILRB1 receptors with HLA class I molecules and the contribution of the bound peptide and its implication from an immunological point of view. In this context, structural immune-informatics approaches have been performed using homology modeling and docking strategies to obtain HLA-B/KIR3DL1 and HLA-B/LILRB1 models that take into consideration the most frequent allele's combination in human population. Models were subjected to energy minimization and analyzed for $\Delta\Delta G$ energy, number of contacts (number of atoms in the interactive surface), and VdW and H-bond, using a combination of utilized public tools. Our results are in agreement with previous findings on HLA/KIRDL1 interaction and can be extended to all the alleles' combination of the study. In HLA/LILRB1 interaction context, we extended our previous evidence for HLA Ile/Val 194 contribution in the interaction with LILRB1. In addition, a contribution of the bound peptide at relative position 1 capable to modulate HLA-B/LILRB1 interaction is hypothesized.

2. Material and Methods

2.1. HLA/KIR3DL1 Interaction Study. HLA-B alleles have been divided depending on carrying Bw4/Bw6 epitope (Bw4: HLA-B*27:05, HLA-B*51:01, HLA-B*57:01, and HLA-B*58:01; Bw6: HLA-B*07:02, HLA-B*14:02, and HLA-B*35:01). HLA supertype [38] and HLA allele frequency in different human population [36] have been considered, as previously reported [18]. KIR3DL1 alleles have been selected depending on human population frequency and coded amino acid polymorphisms (Table 1). Among them, KIR3DL1*015 and KIR3DL1*007 have been analyzed together as they share the same amino acid polymorphisms in the coding region (Table 1). HLA-B peptides with similar EC_{50} (200 nM) were selected as references from IEDB database [39] as previously reported [18].

Table 2: List of LILRB1 haplotypes selected for immune-informatics approach. The LILRB1 haplotypes are distinguished by the following amino acid polymorphisms in the mature protein sequence in accordance with previous classifications [33, 34].

LILRB1 haplotype	LILRB1 amino acid position			
	45	70	119	132
LILRB1.01	L	A	I	S
LILRB1.02	P	T	T	I
LILRB1.03	P	A	I	I

2.2. HLA/LILRB1 Interaction Study. HLA-B alleles have been divided depending on carrying Val or Ile 194 and on their supertype [38], as previously reported [18]. In order to compare different HLA-B alleles interacting with different LILRB1 receptor alleles, we focused our study on the three known haplotypes (LILRB1.01, LILRB1.02, and LILRB1.03) carrying amino acid variants located in amino acid positions 45, 70, 119, and 132 of the mature protein sequence within known LILRB1 alleles and located on the HLA-LILRB1 interaction binding site [33, 34] (Table 2).

2.3. Molecular Modeling and Analysis. Homology modeling strategy has been performed as previously reported [18] and analysis strategy has been schematized as illustrated in the flowchart (Figure 1). Specifically, KIR3DL1 and LILRB1 studies have been performed using the HLA-B57/KIR3DL1*001 (PDB ID: 3HV8) and HLA-A2_LILRB1 (PDB ID: 1P7Q) cocrystallization structures as templates, respectively. MOTIF alignments [40] of the HLA-B structures with the template structures have been performed followed by HLA-B replacement. HLA bound peptide complexes have been generated through single point amino acid substitution (SAS) in the peptide. KIR3DL1 alleles and LILRB1 haplotypes have been obtained through amino acid mutation starting

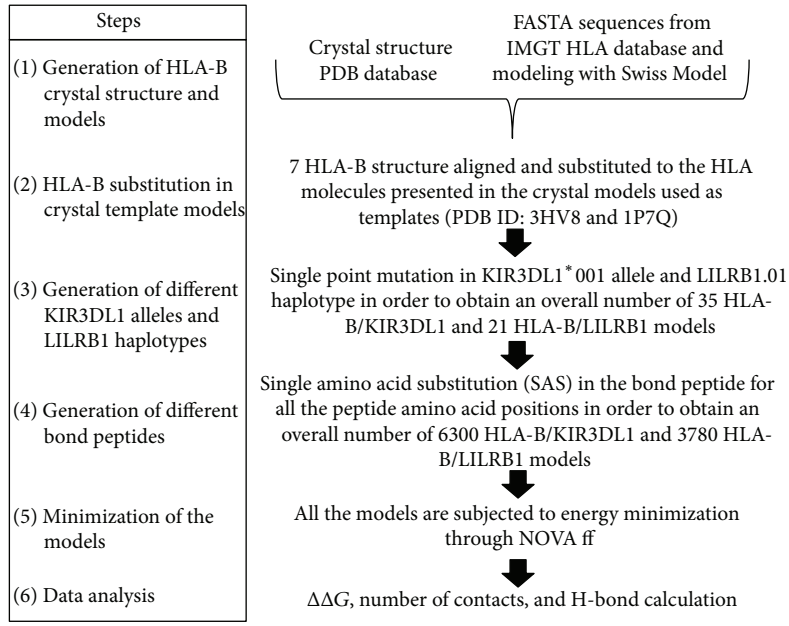

Figure 1: Flowchart to illustrate the analyzing strategy. Schematic procedure for homology modeling study and analysis for both KIR3DL1/HLA and LILRB1/HLA interaction studies.

from KIR3DL1*001 allele and LILRB1.01 haplotype, respectively. A total of 6300 HLA-B/KIR3DL1 and 3780 HLA-B/LILRB1 models have been analyzed. Models were subjected to energy minimization with implemented NOVA force fields (ff) [41].

The receptor binding free energy (ΔG) has been calculated taking into account the interactive energies [18, 42]. The difference in the receptor binding free energy ($\Delta\Delta G$) in presence of different HLA bonds peptides has been calculated as differences between the ΔG of the receptor/HLA peptide (reference) complex minus the receptor/HLA peptide (substituted) complex as previously reported [18, 43].

Statistical analysis and graphs have been performed using the GraphPad Prism 5.0 (San Diego, California). Specifically, Graphics are 10–90 percentiles whiskers and statistical analyses have been performed using the unpaired nonparametric Mann-Whitney test.

Number of contacts has been evaluated using YASARA program and considers the number of atoms interacting in two molecules when calculating interaction energies and instead of considering only atoms closer than a specified cutoff. The higher the number of contacts the higher the strength of interaction. In the context of KIR3DL1 receptor the number of contacts is shown as the sum of the contacts between HLA/KIR3DL1 and peptide/KIR3DL1, while for LILRB1 the number contacts is calculated only between HLA and LILRB1 due to the fact that the crystallography structure used as template contains only LILRB1 D1 and D2 domains which interact only with HLA-B α3 domain and not with the peptide [44].

In addition, the residue-residue contacts map between two chains or within a single one together with the list of the interacting residues has been extracted using the CMA server (contact map analysis) [45].

VdW and solvent surface analysis together with H-bond computation have been calculated as previously reported through WebLab Viewer Pro 3.7 [18].

3. Results

3.1. Contribution of HLA Bw4/Bw6 Epitope with Different KIR3DL1 Alleles. In order to evaluate if carrying Bw4/Bw6 epitope clearly distinguishes the interaction with KIR3DL1 when several alleles' combinations are taken in consideration, KIR3DL1/HLA complexes have been grouped considering Bw4/Bw6 epitope and KIR3DL1 allele (Figures 2(a) and 2(b)). KIR3DL1 alleles are able to interact strongly with HLA-B alleles carrying Bw4 epitope with respect to Bw6 (Figure 2(a)). The difference is highly significant in the case of KIR3DL1*001 (Bw4: mean = 57.9 SD = 274.7, Bw6: mean = −111.5 SD = 317.6; $P < 0.0001$) and *005 alleles (Bw4: mean = 7.5 SD = 285.8, Bw6: mean = −94.9 SD = 306.7; $P < 0.0001$) (Figure 2(a)). A lower but still significant difference is observed in *009 (Bw4: M = −2.8 SD = 310.7, Bw6: M = −57.5 SD = 364.7; $P < 0.005$) and *015/*007 alleles (Bw4: M = 83.7 SD = 299.7, Bw6: M = 30.8 SD = 303.7; $P < 0.005$) (Figure 2(a)). The evaluation of the number of contacts between HLA and KIR3DL1 alleles confirms these results, highlighting also the differences between Bw4/Bw6 epitopes (Figure 2(b)). In this context, binding free energy analysis could have fluctuation due to other energies not directly involved in HLA/KIR3DL1 interaction. Number of contacts mainly take into account the H-bond and VdW energies, which represents the most important features in the HLA/KIR3DL1 interaction according to previous experimental data [11, 14]. For these reasons, due to the great differences observed, the rest of the analyses have been focused only on the number of contacts.

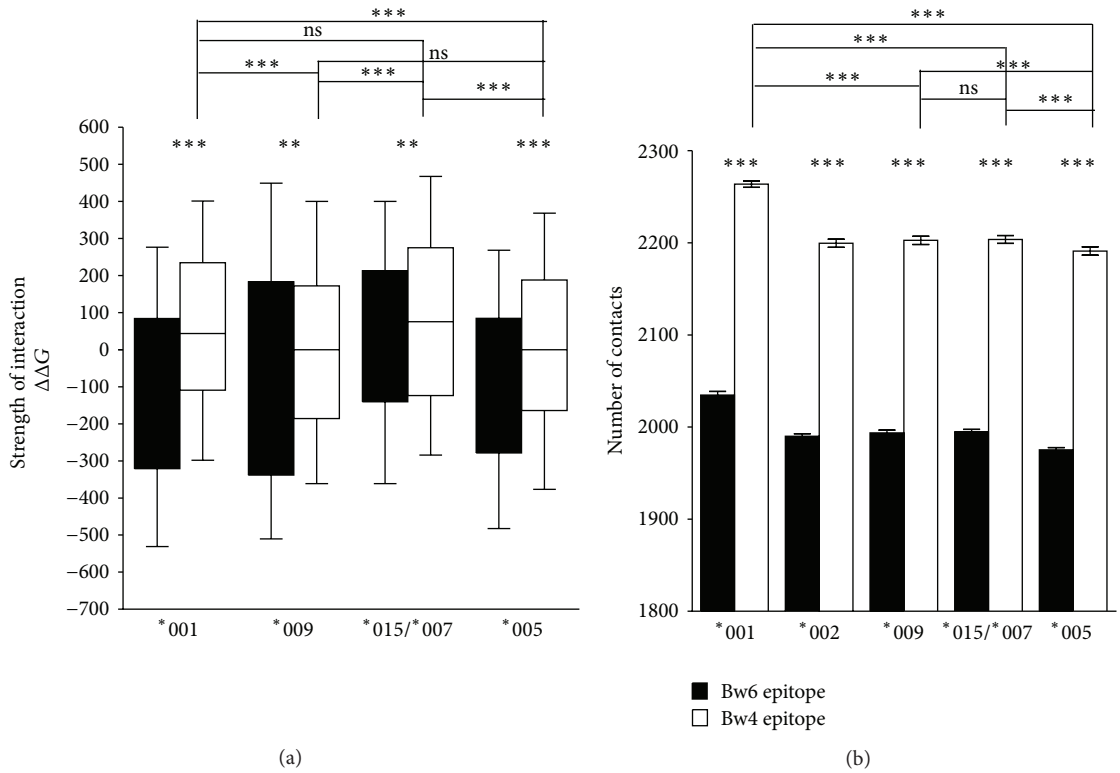

Figure 2: KIR3DL1 alleles and HLA-B allele grouped depending on carrying Bw4/Bw6 epitope. Interaction studies in terms of $\Delta\Delta G$ (a) or number of contacts (b). HLA-B alleles carrying Bw4 epitope (in white) and Bw6 epitope (in black) are shown.

3.2. KIR3DL1 Interaction with Single HLA-B Alleles. In order to evaluate HLA/KIR3DL1 interaction avoiding the interactive differences due to the bound peptide contribution, KIR3DL1 alleles have been studied in combination with HLA-B alleles belonging to the same supertype. In supertype B7 (Figure 3(a)), Bw4 allele HLA-B*51:01 shows the strongest interaction with all the KIR3DL1 alleles of the study followed by the Bw6 alleles HLA-B*35:01 and HLA-B*07:02. The same pattern of interaction observed for supertype B7 is also conserved in supertype B27 (Figure 3(b)). All the KIR3DL1 alleles in supertype B58 show a stronger interaction for HLA-B*57:01 allele with respect to HLA-B*58:01 (Figure 3(c)). Molecular differences in HLA-B/KIR3DL1 alleles' combinations have been considered by performing contact map and H-bond analyses (see Table S1 and Table S2, in Supplementary Material available online at http://dx.doi.org/10.1155/2015/427217). The contact map analysis shows all amino acid residues of HLA and KIR3DL1 molecules with an $\text{Å}^2 > 25.0$ and with different bound peptides with respect to their original crystal structure (Table S1). HLA-B*57:01 and HLA-B*27:05 have the strongest number of amino acid residues involved in H-bond (Table S2).

3.3. Role of the HLA Bound Peptide in HLA-B/KIR3DL1 Interaction. In order to evaluate the contribution of the bound peptide in the interaction, solvent interaction surface peptide/KIR3DL1 has been analyzed (Figure 4). The peptide itself is capable to interact with KIR3DL1 receptor through amino acid position 8 and among the different peptides variants we focused on Glu and Thr variants able to, respectively, disrupt or increase KIR3DL1 binding as previously shown [11]. Amino acid residues in positions 7 and 9 could induce conformational change that modifies the interaction but in less extent with respect to position 8. KIR3DL1 Glu282 has a main role in the peptide recognition. In fact, the negative charge of Glu282 prevents the interaction with negative charged amino acid residues located in peptide position 8. This is a common feature of all the KIR3DL1/HLA-Bw4 combinations analyzed in this study (Figures 4(a)–4(d)). In the same context, noncharged amino acid residues could influence the KIR3DL1 binding depending on the spatial conformation assumed for each HLA peptide binding pocket (Figures 4(e)-4(f)). This is the case of Histidine (His) in position 8 (Figures 4(e)–4(h)). HLA alleles belonging to different supertypes interact with His 8 in a different manner (Figures 4(e) and 4(f)). HLA alleles belonging to the same supertype have a similar pattern of interaction (Figures 4(g) and 4(h)).

3.4. Role of Ile/Val 194 in HLA-B/LILRB1 Interaction. Based on our previous evidences that show a contribution of Ile/Val 194 in LILRB1 interaction [31], we extended the analysis to all the HLA-B alleles of the study dividing them based on Ile/Val 194 (Figure 5). The number of contacts between different HLA-B alleles has been evaluated in LILRB1.01 (Figure 5(a)), LILRB1.02 (Figure 5(b)), and

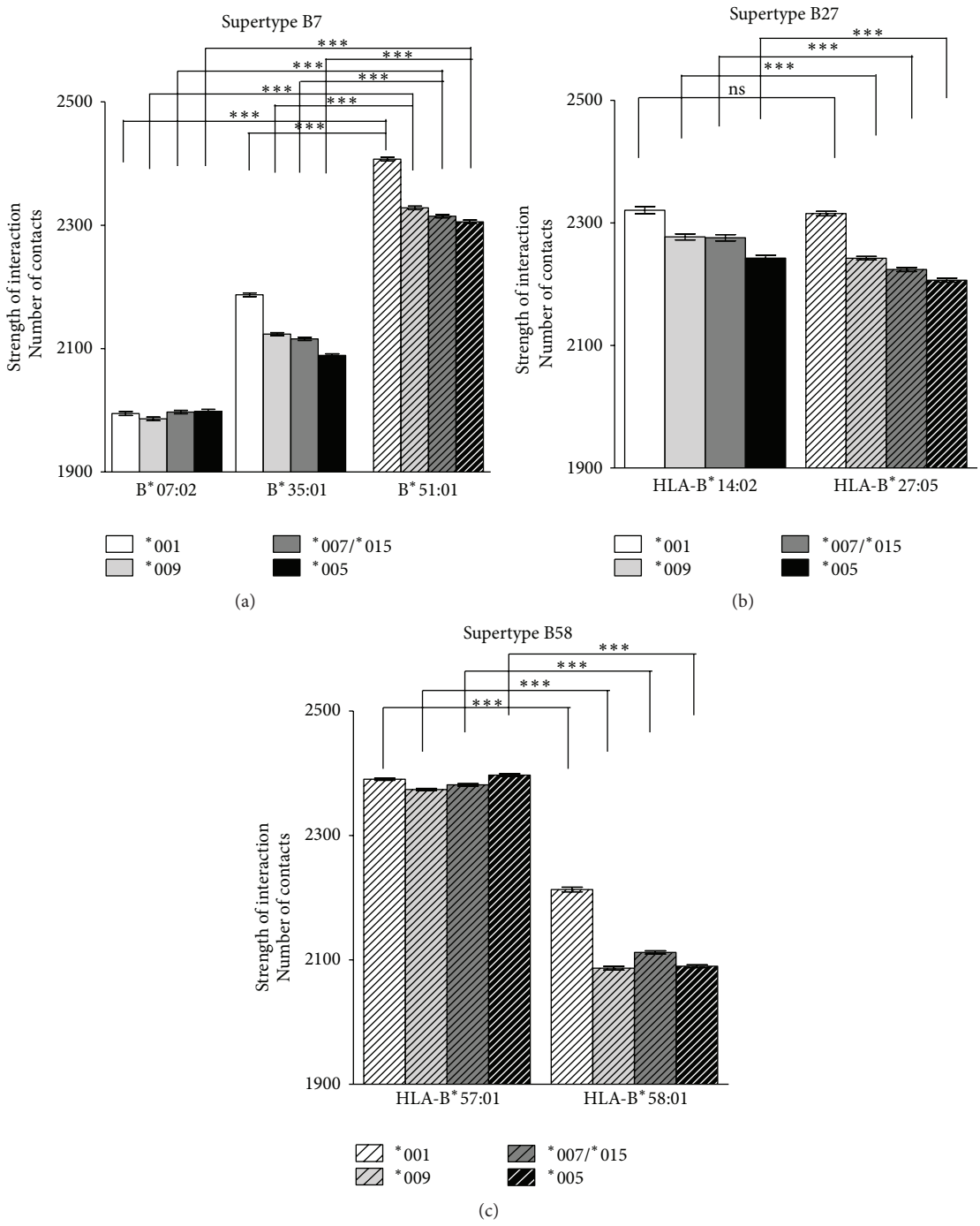

Figure 3: Number of contacts between different KIR3DL1 alleles and HLA-B. HLA-B alleles are grouped depending, respectively, on B7 (a), B27 (b), and B58 (c) supertypes. HLA-B alleles carrying Bw4 epitope (smooth bars) and Bw6 epitope (dot bars) are compared in each supertype.

LILRB1.03 (Figure 5(c)) haplotypes. HLA alleles carrying Ile 194 have a stronger interaction with all the LILRB1 haplotypes with respect to HLA alleles carrying Val 194. This is more evident when HLA-B alleles belonging to the same supertype are analyzed. LILRB1.01 haplotype shows the strongest interaction with HLA molecules followed by LILRB1.02 and LILRB1.03 (Figure 5(d)). Molecular mechanisms of the HLA-B/LILRB1 interaction have been assessed performing H-bond analysis (Table S3). HLA amino acid residues involved in the H-bonds are Asp196, His197, Glu198, and Thr200. LILRB1 amino acid residues involved in the H-bonds are Arg36, Lys 41, Tyr76 and Arg84. We previously reported that the steric hindrance of Ile 194 with respect to Val 194 impacts the site of interaction [31]. In addition to our previous observations, HLA-B alleles carrying Ile 194 have shown the capability to establish more than one H-bond with the same LILRB1

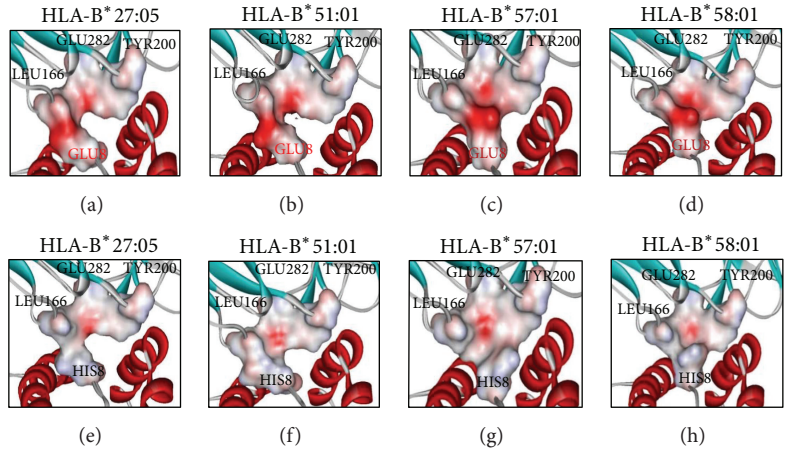

Figure 4: Solvent surface analysis evaluating loaded peptide and KIR3DL1 interaction. The electrostatic potential energy is shown with strongly positively charged (blue), neutral charged (white), and negative charged (red) residues. KIR3DL1 amino acid residues Leu166, Tyr200, and Glu282 and peptide position 8 contacts are shown. Only HLA-B alleles carrying Bw4 supertype are considered in comparison with KIR3DL1 receptor. Peptides Glu8 (a–d) and His 8 (e–h).

amino acid residue with respect to HLA-B alleles carrying Val 194. This capability increases the number of total H-bonds observed in the interaction between LILRB1 and HLA-B alleles carrying Ile 194 with respect to Val 194 (Figure S1). This mechanism is observed in all the HLA-B alleles of the study (data not shown).

3.5. Role of the HLA Bound Peptide in HLA/LILRB1 Interaction. A controversial role in the loading peptide has been shown in the context of LILRB1 interaction. Literature data have not shown a specific peptide position able to modulate the LILRB1 interaction. For these reasons, the contribution of each position of the HLA bound peptide has been investigated. Among them, the amino acid of the HLA bound peptide interacting with pocket A (relative position 1 of the peptide) is capable to interact with HLA-B amino acid position 171 spatially closer to LILRB1 binding site. This suggests that the amino acid variants in relative position 1 of the peptide could induce a conformational change in HLA α_3 domain, therefore affecting the HLA/LILRB1 site of interaction. HLA-B*27:05 allele has been analyzed due to the previous experimental results demonstrating its interaction with LILRB1 [46] and for its extensively studied MHC binding pocket capability. In particular HLA-B*27:05 has been evaluated only for P1 residues with a positive score in the MHC motif Viewer matrix and therefore capable to be bonded in pocket A [47]. The presence of Arginine in pocket 1 induces the strongest number of contacts in HLA-B*27:05/LILRB1.01 haplotype interactions and the presence of Methionine has the lowest. The difference between these two residues is conserved in all the LILRB1 haplotypes even if different trends of interaction can be observed in the context of the other amino acid residues (Figure S2). HLA-B*27:05/peptide/LILRB1 complex carrying either Arginine (Arg) or Methionine (Met) in peptide position 1 (P1) has been further analyzed (Figure 6). Amino acid residues Arg or Met in P1 induce a conformational change from HLA position 171 to HLA loop of 196–200 (Figure 6(a)). The presence of Arg P1 induces a conformational change in HLA His 197, reducing its distance with LILRB1 Tyr38. This increases the contact surface between HLA and LILRB1 (Figure 6(b)). In presence of Met P1, a strong conformational change in the entire HLA molecules is observed. Consequently, the distance between HLA His 197 and LILRB1 Tyr 38 increases (Figure 6(c)).

4. Discussion

Several studies evaluate the different contribution of KIR3DL1/HLA-B allele's combination which could therefore modify the innate immune system modulation [8–11, 14, 16]. In this context, for the first time we performed extensive interaction analyses considering the most frequent HLA-B/KIR3DL1 alleles' combination in the human population. The presence of HLA-B Bw4 epitope leads to a stronger interaction with all the KIR3DL1 alleles with respect to HLA-B Bw6 epitope. When HLA-B alleles belonging to the same supertype are analyzed, the difference is more evident as previously reported [6, 18, 48, 49]. KIR3DL1*001 shows the stronger interaction with HLA molecules in terms of number of contacts. The same interaction is also observed in the KIR3DL1 high expressive alleles *009 and *015. On the contrary, a reduced number of contacts is observed in the KIR3DL1 low expressive *005 allele which leads to the conclusion that this particular allele might have a weaker interaction with HLA molecules. These results are in agreement with previous studies performed on HLA-B*51:01 and its interaction with several KIR3DL1 alleles in which a weaker binding of the low expressive *005 allele is shown [10]. Different association studies show that KIR3DL1 high expressive alleles are associated with a delayed HIV infection progression [7]. In this study we show that behind the NK cells' surface expression they also have a stronger interaction with Bw4 epitope. Most of the contacts between HLA-B and KIR3DL1 alleles are due to VdW interaction and H-bonds that are specific for each HLA-B allele. In this context, even if

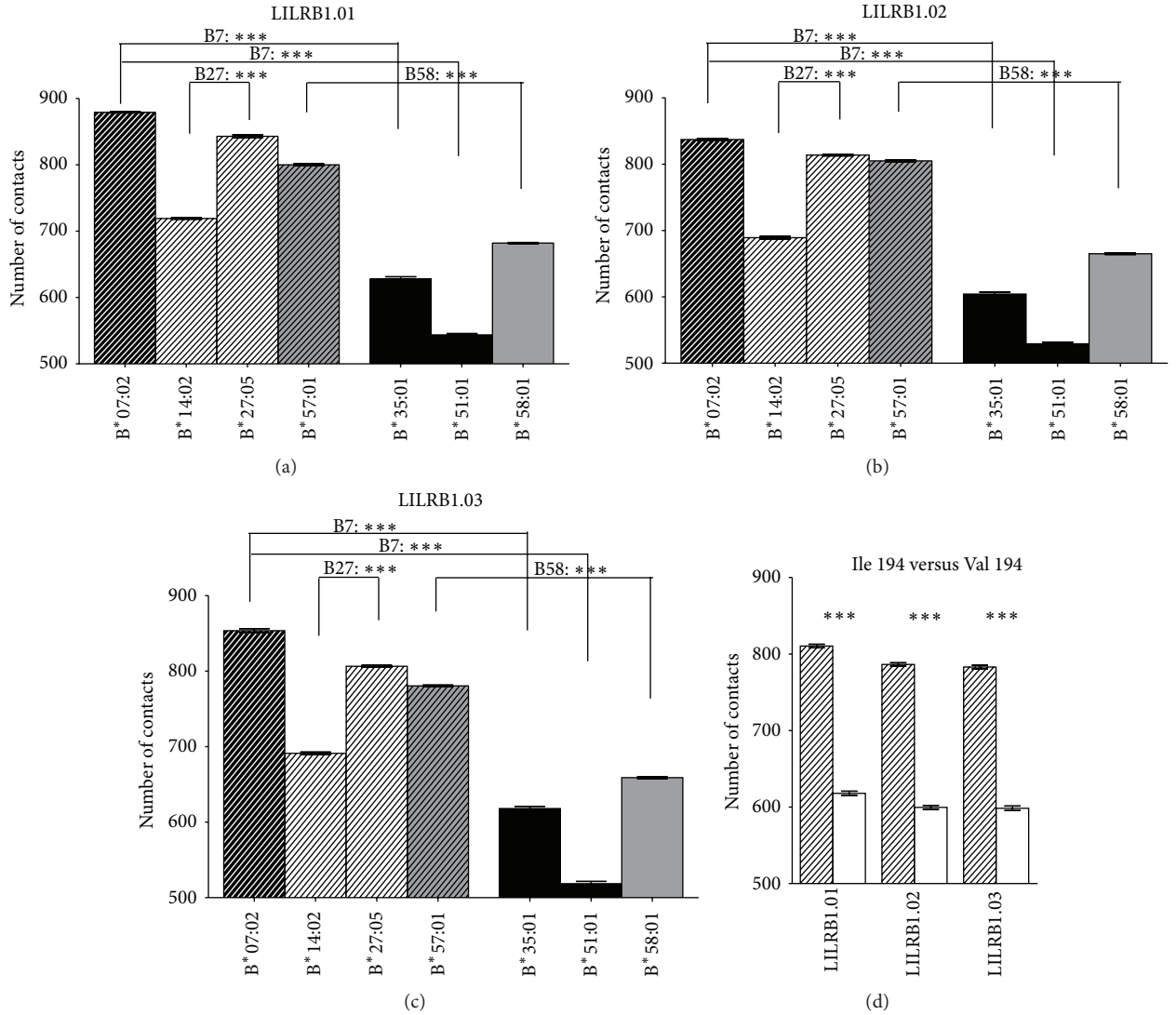

Figure 5: Analysis of number of contacts between different LILRB1 haplotypes and HLA-B alleles. HLA-B alleles interacting with LILRB1.01 (a), LILRB1.02 (b), and LILRB1.03 (c) haplotypes and grouped depending on Ile/Val 194 polymorphisms (d) are shown. For each LILRB1 haplotype, HLA-B are divided depending on Ile 194 (line bars) or Val 194 (smooth bars) and comparison of HLA-B alleles belonging to the same supertype is performed. HLA-B alleles belonging to B7, B27, and B58 supertypes are shown, respectively, in black, white, and grey.

HLA-B*14:02 allele carries Bw6 epitope, it presents a higher number of H-bonds with KIR3DL1 that might be evidence for a weak binding with KIR3DL1 receptor. This result also suggests that other amino acid residues behind Bw4/Bw6 epitope could influence the HLA-B/KIR3DL1 interaction network. Finally, the contribution of the peptide seems to be the key point able to disrupt HLA-B/KIR3DL1 interaction. In particular, negative charged amino acid residues in peptide position P8 disrupt the KIR3DL1 binding. This is due to a charge repulsion with the conserved amino acid residue Glu282 of KIR3DL1 as previously observed [11, 14–17, 35, 50]. On the contrary, the capability of KIR3DL1 binding due to other amino acid residues located in peptide position P8 depends also on the HLA-B allele and does not follow general rules. Future studies are needed in order to associate the presence of Glu in peptide P8 with an increase of NK cells' cytotoxic immune response through the lack of KIR3DL1 binding. In this context, the use of viral peptides with Glu P8 should be capable to increase NK cells' cytotoxic immune response, therefore exerting a protective role in the disease progression.

The contribution of LILRB1 interaction with HLA class I particularly in the context of several infections has been already assessed [27, 30, 51]. Among HLA class I polymorphisms, we previously associated amino acid position 194 of HLA-B with different HIV progression [31]. In this study, a larger set of HLA-B alleles carrying Val/Ile 194 have been studied using immune-informatics approaches. These types of approaches are important to evaluate a large set of data and to hypothesize the general rules of interaction. Specifically, the contribution of amino acid position 194 polymorphisms in the interaction with different LILRB1 haplotypes has been evaluated. HLA-B alleles carrying Ile 194 polymorphism show a higher strength of interaction with respect to HLA-B

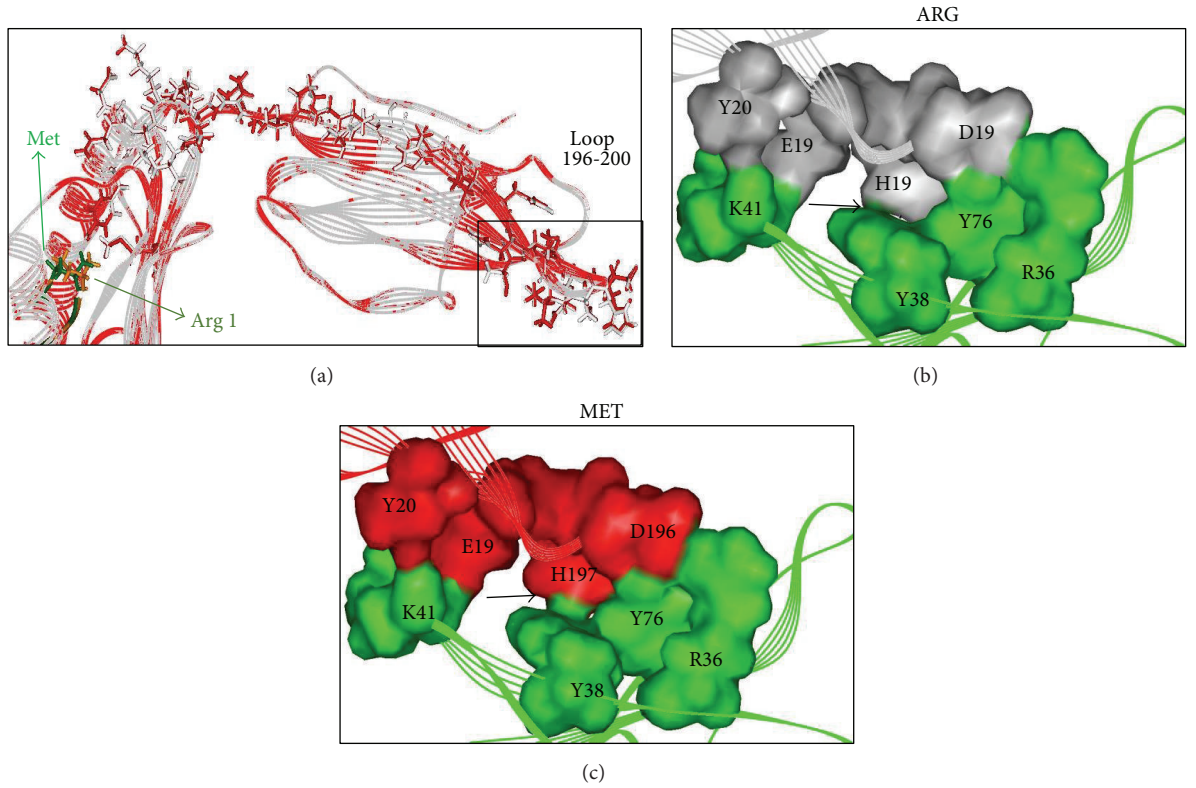

Figure 6: Molecular homology modeling of interaction site between HLA-B*27:05 and LILRB1.01 haplotype. (a) Alignment of HLA-B*27:05 molecules carrying, respectively, Arginine (gray) or Methionine (red) in peptide position 1. Interaction between LILRB1.01 haplotype (in green) and HLA-B*27:05 in presence of Arginine (b) or Methionine (c) in peptide position 1.

alleles carrying Val 194. This pattern is observed in all the LILRB1 haplotypes analyzed in terms of number of contacts as well as H-bonds in the site of interaction. These results are in agreement with recent studies showing an influence of allelic variation and conformation of HLA class I on LILR binding [30, 51]. A stronger interaction of LILRB1.01 haplotypes with HLA-B alleles is shown with respect to LILRB1.02 and LILRB1.03 haplotypes. This evidence is consistent with the affinity constant retrieved after the interaction studies between HLA-B*35:01 and the three LILRB1 haplotypes [34]. Finally, for the first time we indicate a contribution of amino acid variants located in HLA bound peptide at relative position 1, capable to modulate HLA-B/LILRB1 interaction. This evidence is in agreement with previous results showing different binding capability of LILRB1 with HLA-B*27:05 in the presence of different HLA bound peptides [35]. Different questions about LILRB1 interaction still need to be addressed. In this context, future experiments are needed to evaluate the contribution of HLA class I Ile/Val 194 polymorphism in LILRB1 interaction. Together with these issues, the contribution of peptide polymorphisms could also affect the LILRB1 binding and it still needs to be addressed.

Beside the similar inhibitory function exerted by KIR3DL1 and LILRB1 receptors and the fact that they both recognize HLA class I molecules as common ligand, several differences can be observed from a structural point of view. KIR3DL1 recognize mainly α_1 and α_2 domains of HLA molecules characterized by extreme variability in human population with a strong contribution of Bw4/Bw6 peptide located in proximity of the F pocket. LILRB1 mainly interact with HLA α_3 domain, a higher conserved region of HLA class I molecules. Interestingly, HLA bound peptide can exert itself to a contribution in KIR3DL1/LILRB1 interaction even though KIR3DL1 is affected by amino acid residues in peptide position 8 while LILRB1 modifies its strength of interaction depending on amino acid residues in peptide position 1.

The homology models analyzed in this study represent a starting point for driving these future experiments and shed new light on the contribution of different peptide positions in driving the immune response.

Overall, the strong influence of the bound peptide in innate immune response points out similarity between T-cell and NK cells. It has been suggested that individual selection pressures exerted on HLA class I by T-cell and NK cell immunity can compete with each other. Thus, depending on the variant HLA class I and the peptide presented a beneficial NK cell response with detrimental consequences for T-cell response or vice versa might be observed [7]. In this context, we propose a common mechanism in the activation of NK cell cytotoxic immune response behind the "missing self" hypothesis. Specifically, the presence of negative amino acid charged residues in peptide P8 is able to disrupt KIR3DL1 binding and leads to NK cell immune response. This mechanism is not so clear in the context of HLA/LILRB1 interaction even though it seems also to depend

on peptide variants. In conclusion, the bound peptide in HLA class I molecules is the key regulator factor capable to drive both innate and adaptive immune responses.

Conflict of Interests

The authors declare that they have no commercial or any other conflict of interests regarding the presentation of the data reported in the paper.

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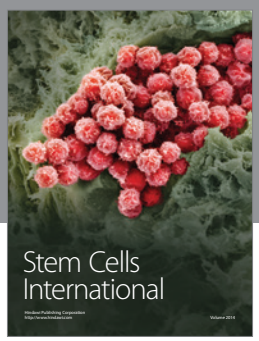

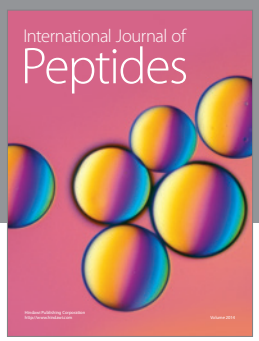

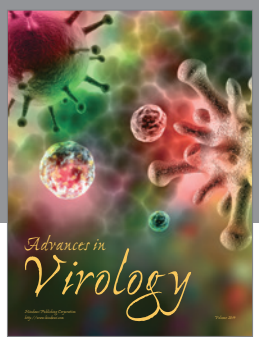

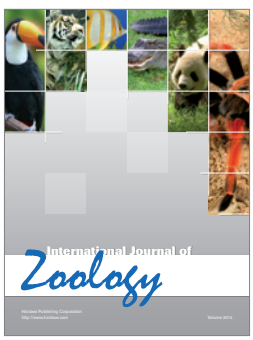

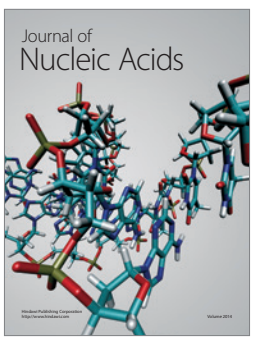

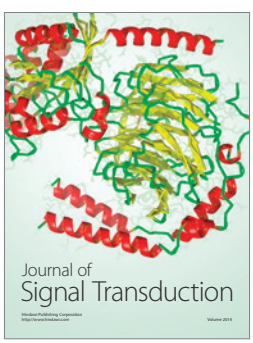

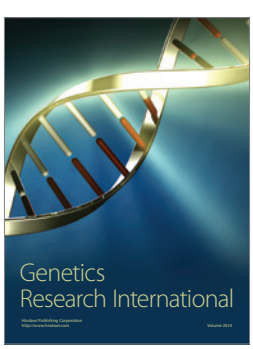

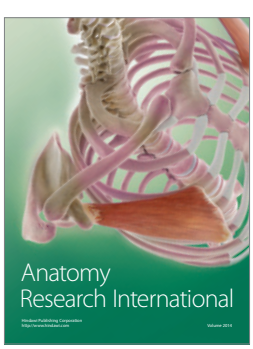

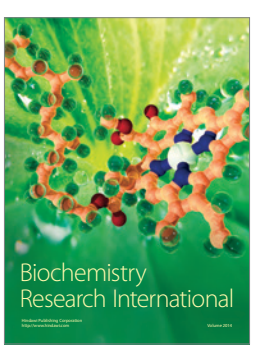

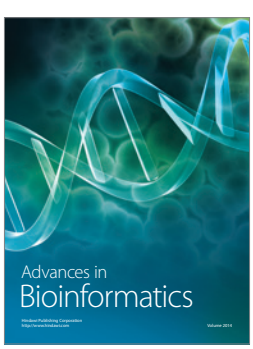

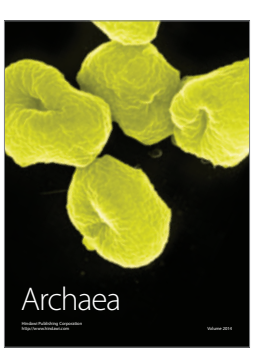

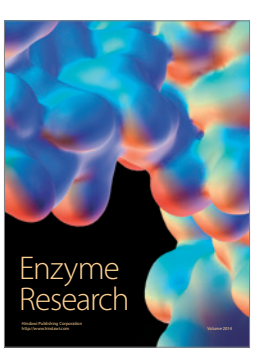

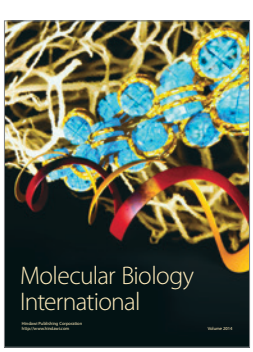

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