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How a single amino acid change may alter the immunological information of a peptide

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TABLE OF CONTENTS

1. ABSTRACT

What types of amino acid substitutions are functionally tolerated in an epitope? This question is of importance because the immunogenicity, pathogenicity, and therapeutic potential of a peptide can be determined by a single amino acid change. As an example, a single amino acid change in the encephalitogenic myelin oligodendrocyte glycoprotein WYRSPFSRVV peptide confers the capacity to ameliorate and reverse experimental autoimmune encephalomyelitis. Currently, no rule is available to predict/explain the functional outcomes of amino acid changes. To address this issue, we examined the role of single amino acid changes in immune responses by applying proteomic similarity analyses to available data. We found that the loss or gain of immunological information in a peptide epitope following an amino acid substitution often is related to a gain or loss in the proteomic similarity. Rare, but significant epitopic sequences become immunologically insignificant when an amino acid change makes them common, repeated sequences. This study confirms that low similarity to the host proteome is a major factor in modulating the immune epitope repertoire.

2. INTRODUCTION

The functional importance of even a single amino acid (aa) substitution is evident at both the physiological and pathological levels. For example:

- Single aa changes confer constitutive activation of mTOR, a serine/threonine kinase that regulates a variety of cellular functions such as growth, proliferation, and autophagy and is also overactivated in cancer cells (1).

- One aa change ($Gly^{269}Ser$) in the S5 segment of the voltage-gated cardiac ion channel, KvLQT1, leads to long QT syndrome, which is an arrhythmic disorder (2).

- Cell adhesion and motility in living cells is altered by a single aa change in E-cadherin (3).

- In humans, one variant of the catestatin peptide, ^{352}SSMKLSFRARGYS^{364}FRGPGPQL^{372} with $Gly^{364}Ser$, profoundly alters autonomic activity in both the parasympathetic and sympathetic branches of the nervous system and protects against the future development of hypertension (4).

- a single aa change ($Lys^{141}Asn$) can allow a chimeric Theiler's virus to persist and demyelinate (5).

Most importantly, the degree of freedom for aa substitutions is the lowest in immunology. In general, a single aa substitution may have profound effects on immune responses and recognition. For example:

- A synthetic peptide containing a single aa change (Leu >His) was more than l000-fold more stimulatory to T cells than the wild-type peptide (6).

- A Ile-to-Phe substitution in the SCILLYIVI nonapeptide defines the antigenic polymorphism and T-cell specificity of the murine H47 locus (7).
- Myelin oligodendrocyte glycoprotein (MOG) is a myelin target antigen, and MOG peptides have been shown to have encephalitogenic activity. Strikingly, an Ala substitution at position 44 in the $MOG_{39–48}$WYRSPFSRVV peptide almost totally abrogates (96% reduction) the capacity to stimulate encephalitogenic line T cells (8); and

- a $Lys^{149}Arg$ mutation in the antigenic site of hemagglutinin can alter the recognition of H9N2 influenza virus by a monoclonal antibody (MAb) (9).

However, when aa changes are analyzed, the specific link between aa substitutions and altered immuno-biological function(s) remains elusive. For example:

- Presenilin-1 is a subunit of the gamma-secretase complex, and, when altered, is involved in Alzheimer disease type 3. It has been reported that an aa substitution of Glu for Asp at position 385 abolishes gamma-secretase activity. Likewise, a change in the hydrophobic, bulky, aromatic acid (Tyr) to another hydrophobic, bulky, aromatic acid (Phe) at position 256 alters gamma-secretase cleavage specificity (10, 11). In contrast, changing the negatively charged Asp to the positively charged Asn at position 373 has no effect on caspase cleavage (11). In summary, it seems that maintaining the acid (or hydrophobic) features does not protect the functional characteristics, whereas changing from an acidic to a basic residue does not affect the function.

- Even changes directed towards the identical aa may affect function. Golgi coiled-coil protein GCC185 (also called renal carcinoma antigen NY-REN-53) was characterized following mutagenesis of Leu/Tyr to Ala with two opposite outcomes. Specifically, an aa substitution of Ala for Leu at position 1494 ($Leu^{1494}Ala$) decreases the RAB6A binding affinity two-fold, strongly decreases the RAB9 binding affinity, and abolishes Golgi localization. However, an aa substitution of Ala for Tyr at position 1517 ($Tyr^{1517}Ala$) has no effect on RAB6A or RAB9 binding (12).

- Tuberin aa $Arg^{611}Gln$, $Arg^{611}Trp$, $Ala^{614}Asp$, $Phe^{615}Ser$, $Cys^{696}Tyr$, and $Val^{769}Glu$ substitutions inhibit the phosphorylation of tuberin by the Ser/Thr protein kinase B (PKB, also known as Akt) (13). Specifically, disparate substitutions - i.e., introducing basic, hydrophobic, acid, or polar residues - produce the same final outcome in inhibiting tuberin phosphorylation, a pathogenic reaction that disrupts tuberin-hamartin interactions.

- Further paradigmatic examples occur in the polymorphic epithelial mucin (also called episialin or breast carcinoma-associated antigen DF3), which is an antigen that is overexpressed in tumors and shows cell-adhesive properties and participates in cell signalling. Mutagenesis experiments involving the same aa change in five different mucin positions produced a range of functional outcomes. More specifically, changing Tyr to Phe i) has no effect on EGFR-mediated phosphorylation at aa positions 1191, 1203, and 1218 (14–16); ii) causes some reduction in EGFR-mediated phosphorylation at aa position 1209 (14); and iii) greatly reduces EGFR- and Src-mediated phosphorylation at aa position 1229 (14, 17, 18).

Likewise, physico-chemical parameters are not a stringent enough criterion to predict/evaluate the ability of aa changes to affect the immunogenic potential of a peptide sequence:

- The H13a and H13b alleles encode for the minor histocompatibility antigen (mHAgs), which are presented in the context of the $H2D^b$ MHC class I molecule. These alleles are pathologically important because they evoke reciprocal T-cell responses in H13a and H13b congenic strains. The molecular basis of the reciprocal T-cell responses between the two mHAg alleles resides in a conservative single aa substitution (Val/Ile) (19–22).

- A single Leu residue at position 1858 in the helicase domain of the pestivirus NS3 protein is critical for the genesis of an epitope recognized by the MAb P1D8. Changing the aliphatic Leu to aliphatic Val ($Leu^{1858}Val$) or polar Ser ($Leu^{1858}Ser$) destroys the NS3 epitope (23). That is, the maintenance of aliphatic characteristics or the acquisition of a polar feature results in, but does not explain, the same event: epitope destruction.

The data cited above raise an important question; how can a single aa change cause a gain/loss in the immunological content of a peptide sequence? During the last decade, Kanduc (24–27) reported data suggesting that the level of similarity to the host proteome modulates the immunogenicity of a peptide sequence. Kanduc hypothesized that the immunological information contained in an amino acid sequence is directly related to its rarity (28). As an extension of these studies, the present investigation explores whether there may be a link between single aa substitutions, sequence-similarity changes, and altered antigenicity/immunogenicity of a peptide.

3. METHODS

Published studies on single aa substitutions in peptide sequences were obtained from PubMed (http://www.ncbi.nlm.nih.gov). Peptide sequences were analyzed for similarity changes using the Protein

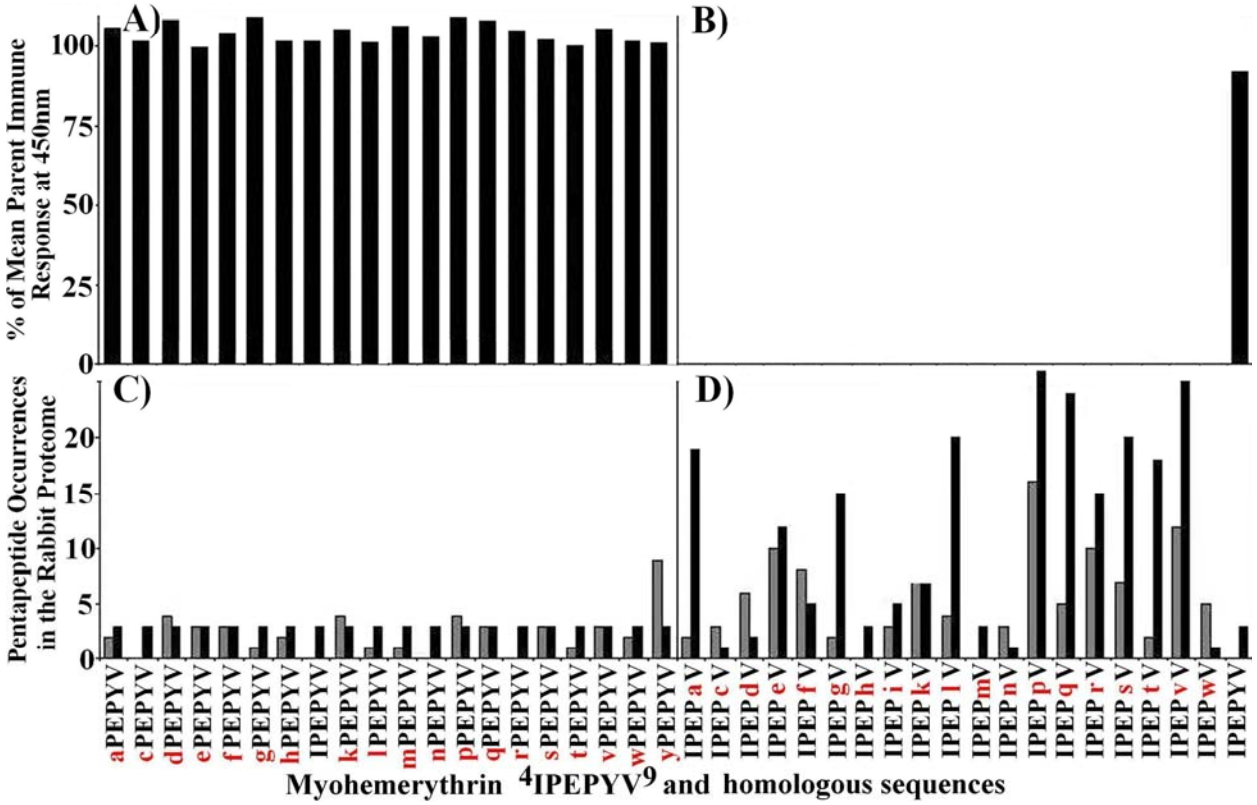

Figure 1. Relationship among single aa changes, antigenicity shift and peptide rarity: analysis of the antigenic myohemerythrin sequence ^{4}IPEPYV^{9}. Upper panels: reaction with rabbit antibody of a complete replacement set of peptides in which all 20 amino acids were substituted in turn at Ile^{4} (A) or Tyr^{8} position (B) within the antigenic IPEPYV sequence. Data from refs. 43 and 44. Lower panels: similarity analysis at the pentapeptide level of hexamers derived by aa substitution at Ile^{4} (C) or Tyr^{8} (D) position within the antigenic IPEPYV sequence. Substituted hexamers were dissected in two overlapping pentapeptides shifted by one residue (eg, aPEPY and PEPYV, indicated by grey and black bars, respectively). Each pentapeptide was analyzed for matches in the rabbit proteome as described under Methods. The columns indicate the pentapeptide proteomic similarity quantified as number of pentapeptide occurrences in the rabbit proteome. Substituted aa in red lowercase.

Information Resource perfect match program (www.pir.georgetown.edu) (29). The rabbit proteome was downloaded from www.ensembl.org. Peptides were dissected into pentapeptides shifted by one residue and then each pentapeptide was analyzed for exact matches in the host proteome. The pentapeptide was used as a basic unit because a grouping of five residues represents a sufficient minimal antigenic determinant of immune responses and recognition (24, 30–35). Pentapeptide similarity is defined by the number of exact pentameric matches in the host proteome (24, 36), and can range from zero to hundreds (37). Based on previous data (38–42), a pentapeptide fragment that has about five perfect matches to the host proteome can be considered a low-similarity pentapeptide, i.e., a rare fragment.

4. RESULTS

4.1. Single amino acid changes, proteomic similarity, and immune recognition

In studying the particular epitope amino acids conferring specificity for the reaction with an antibody, Getzoff *et al.* (43, 44) demonstrated that substituting all 20 amino acids at the Ile^{4} and Tyr^{8} positions within the antigenic myohemerythrin$_{4-9}$IPEPYV sequence exerts a differential effect on peptide antigenicity (Figure 1). Specifically, the authors found that Ile^{4} was replaceable (Figure 1, panel A), whereas Tyr^{8} was essential for immune reactions with rabbit antisera (Figure 1, panel B). Indeed, data from Panel A appear to indicate that the epitope actually corresponds to the pentapeptide PEPYV. We explored the sequence similarity of the homologous epitope sequences versus the rabbit proteome and found that aa changes that do not alter the low similarity level of the epitope (Fig 1. panel C) do not change its antigenicity potential (Figure 1, panel A). Conversely, with few exceptions, aa changes leading to an altered epitope similarity level (Figure 1, panel D) are associated with altered antigenicity potential (Figure 1, panel B).

The relationship between a single aa change, an antigenicity shift, and peptide rarity appears evident also when different residues in an epitope are substituted with an identical residue, as shown in Ala scanning experiments. Recently, Hjelm *et al.* (45) defined a sequence of 11 amino acids (RIERATGQRPH) as the core epitope of a MAb against human tryptophanyl-tRNA synthetase, and found

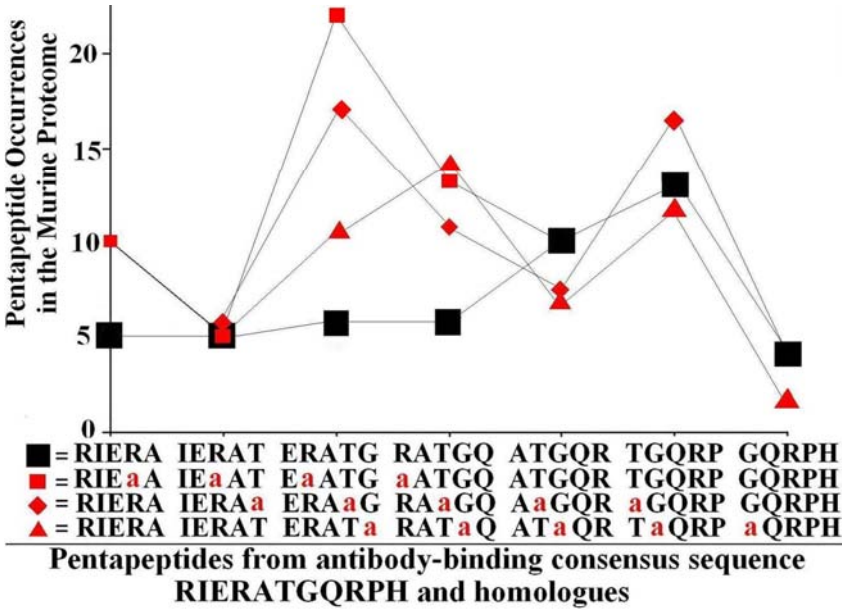

Figure 2. Relationship among Ala substitutions, antigenicity shift and peptide rarity: similarity profile of the antigenic sequence RIERATGQRPH and its Ala-substituted homologues versus the murine proteome at the pentapeptide level. Pentapeptides forming the MAb-binding peptide RIERATGQRPH and its homologues are reported along the X axis. MAb-binding sequence: RIERATGQRPH (black squares). Homologous sequences: RIEaATGQR (red squares); RIERAaGQR (red triangles); RIERATaQR (red diamonds). Ala substitutions in red lowercase. RIERATGQRPH and its Ala-substituted homologues were dissected into pentapeptides overlapping by four amino acids; then, each pentapeptide was analyzed for matches in the murine proteome as described under Methods. The columns indicate the pentapeptide proteomic similarity quantified as number of pentapeptide occurrences in the murine proteome.

that homologues derived from Ala substitutions in the epitopic sequence (RIEaATGQR, RIERAaGQR, and RIERATaQR, with Ala substitutions in lowercase) did not bind to the antibody. Our similarity analyses of the binding epitope RIERATGQR and its non-epitopic homologues (Figure 2) show that Ala substitution transforms the low-similarity central pentapeptides into high-similarity homologous sequences. That is, loss in sequence antigenicity appears to be accompanied by a gain in proteomic similarity.

Analogous data are reported in Figure 3, which illustrates the similarity profiles of the Denque virus ^{101}WGNGCGLFG^{109} epitope and its homologue ^{101}WGNGgGLFG^{109} sequence containing a Cys^{105}Gly change (given in lowercase), versus the murine proteome. It can be observed that a substitution of a C with a G transforms the low-similarity sequence (Figure 3, panel A) into a high-similarity sequence (Figure 3, panel B). Again, it is worth noting that Dengue reactive mAbs react more strongly with the flavivirus-conserved low-similarity ^{101}WGNGCGLFG^{109} fusion sequence than with the high-similarity homologue (46, 47).

A similar picture emerges from the analysis of a single aa substitution in the five core residues (^{71}AGNVN^{75}) of the immunodominant mycobacterial epitope p61–80/PT19 (^{61}VTGSVVCTTAAGNVNIAIGG^{80}) (45). An Asn^{73}Ala change abolishes T immune recognition of the target epitope and, concomitantly, changes the proteomic similarity level of the five core residues from one match (^{71}AGNVN^{75}) to eight matches (^{71}AGaVN^{75}, with the Asn^{73}Ala substitution given in lowercase) (32, 48).

Alexander *et al.* also reported data linking changes in similarity level to changes in immunological information (49). These authors found that peptides differing in only 1 of 19 amino acids (Q versus R) are able to induce allospecific antisera. Specifically, Thy1.2 peptide 88 (KDEGDYFCELQVSGANPMS) elicits rabbit antisera recognizing only the Thy1.2 allotype, whereas Thy1.1 peptide 90 (KDEGDYFCELrVSGANPMS, with the Q^{89}R change in lowercase) elicits rabbit antisera recognizing Thy1.2 and Thy1.1 allotypes (46, and further details therein). Of note, the conservative aa substitution (Q versus R) changes the pentapeptide CELQV (characterized by two matches in the rabbit proteome) into the pentapeptide CELrV (occurring six times in rabbit proteins). In other words, the loss in immunological allospecificity appears to be associated with a gain in proteomic similarity.

4.2. Single amino acid changes, proteomic similarity, and immunogenicity/ pathogenicity

In Lewis rats, the peptide ^{273}TWEGSGVLPCV^{283} of the rat interphotoreceptor retinoid-binding protein (IRBP) causes experimental autoimmune uveoretinitis (EAU) at 200 nmol/rat (50). A homologous peptide, in which residues 277 and 282 (i.e., terminal sequence residues in which MHC binding potential is allocated), were substituted with V and D, respectively, drastically

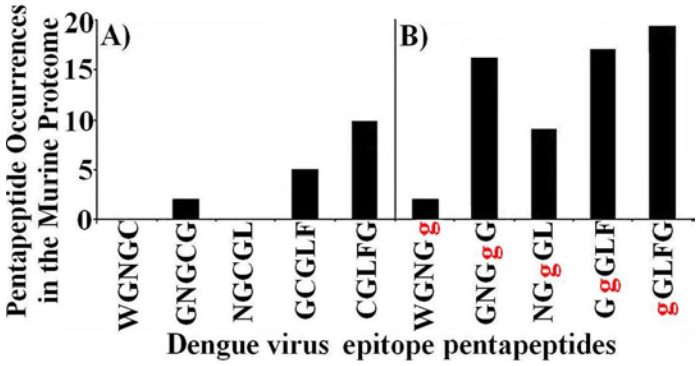

Figure 3. A Gly substitution at 105 position transforms the reactive low-similarity Dengue virus fragment ^{101}WGNGCGLFG^{109} (panel A) into a less reactive high-similarity fragment ^{101}WGNGgGLFG^{109} (panel B). The Cys^{105}Gly substitution is given in red lowercase. Data on epitope (non)reactivity from refs. 46 and 47. Peptide sequences were dissected into pentapeptides overlapping by four amino acids; then, each pentapeptide was analyzed for matches in the murine proteome as described under Methods. The columns indicate the pentapeptide proteomic similarity quantified as number of pentapeptide occurrences in the murine proteome.

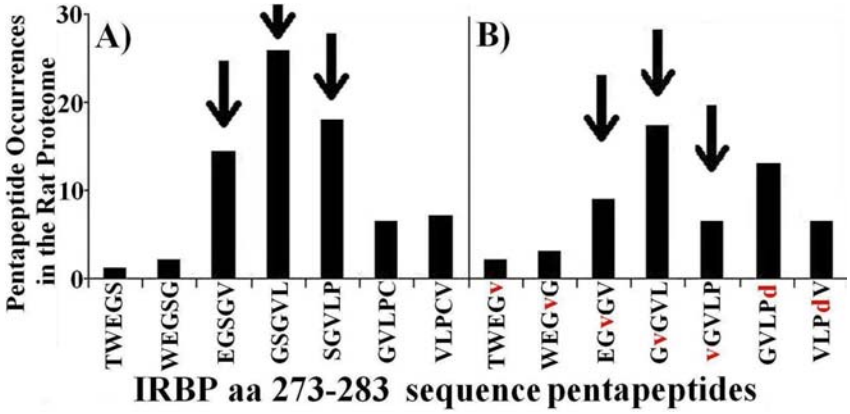

Figure 4. Sequence similarity analysis of pentapeptides forming the IRBP ^{273}TWEGSGVLPCV^{283} (A) and homologous ^{273}TWEGvGVLPdV^{283} (B) peptides. Substitutions are given in red lowercase. Peptide sequences were dissected into pentapeptides overlapping by four amino acids; then, each pentapeptide was analyzed for matches in the rat proteome as described under Methods. The columns indicate the pentapeptide proteomic similarity quantified as number of pentapeptide occurrences in the rat proteome. Arrows highlight the proteomic similarity change of the central pentapeptides.

increased (i) MHC affinity; and (iii) pathogenicity, by causing disease even at 0.02 nmol/rat. In addition, Figure 4 shows that the pentapeptide similarity profiles of IRBP epitope ^{273}TWEGSGVLPCV^{283} (Figure 4, panel A) and its homologue ^{273}TWEGvGVLPdV^{283} (Figure 4, panel B, with substitutions given in lowercase) are different. Specifically, the Ser^{277}Val substitution decreases the proteomic similarity level of the epitopic central pentapeptides (see arrows in Figure 4), in which the epitope immunogenicity lies (32, 51). Consistent with the data patterns discussed above, Figure 4 appears to indicate a link between the decreased similarity of the core pentapeptides and the increased peptide immunogenicity.

4.3. Single amino acid changes, proteomic similarity, and therapeutic peptides

No less importantly, single aa substitutions can generate immunotherapeutic peptide analogues. Caspi *et al.* (52) described two peptide analogues derived from a major uveitogenic epitope, ^{161}SGIPYVISYLHPGNTVMHVD^{180}, from the murine retinal antigen IRBP, which were able to protect against antiretinal autoimmunity. The two inhibitory homologous peptides, namely 169A and 171A, differed from the pathogenic parent by single Ala substitutions at positions 169 and 171, respectively. In applying our similarity rationale, we expected an increase in the sequence-similarity score of the two protective peptides as compared to the pathogenic precursor. Figure 5 confirms this hypothesis and shows that Ala substitution at position 169 or 171 causes an increase in the proteomic similarity of the uveitogenic epitope sequence (Figure 5, panels B and C, respectively) as compared to the pathogenic peptide (Figure 5, panel A).

Likewise, a single amino substitution marking the transformation of the encephalitogenic $MOG_{39–48}$WYRSPFSRVV peptide into the non-encephalitogenic $MOG_{39–48}$WYRSPaSRVV peptide (8, 53) is accompanied

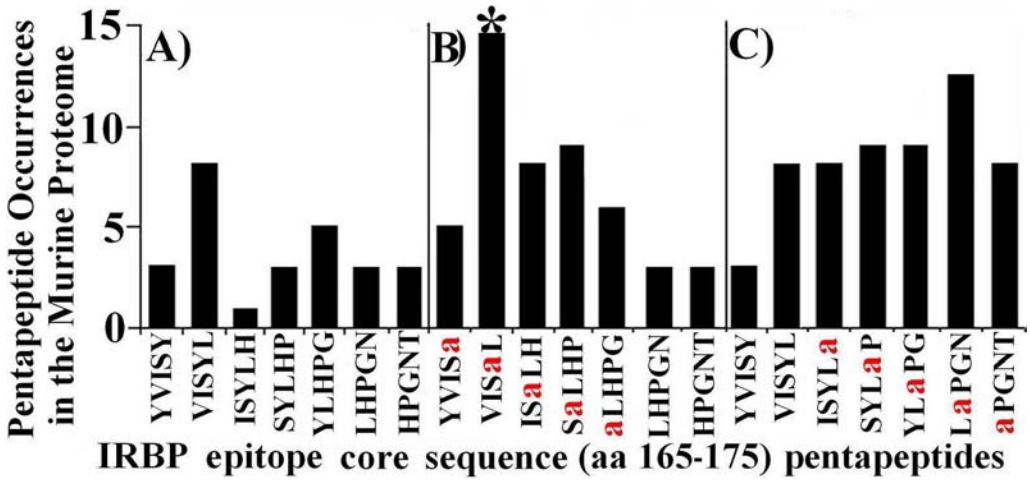

Figure 5. Sequence similarity analysis of pentapeptides forming the core of a major uveitogenic epitope, ^{165}YVISYLHPGNT^{175} from the murine retinal antigen IRBP (panel A); the homologous protective peptide 169A hosting Tyr^{169}Ala substitution (panel B); the homologous protective peptide 171A hosting His^{171}Ala substitution (panel C). Substituted aa given in red lowercase. The uveitogenic epitope and its homologues were dissected into pentapeptides overlapping by four aminoacids; then, each pentapeptide was analyzed for matches in the murine proteome as described under Methods. The columns indicate the pentapeptide proteomic similarity quantified as number of pentapeptide occurrences in the murine proteome. In panel B, the asterisk refers to the pentapeptide VISaL, which occurs 43 times in the murine proteome.

by a change in the proteomic similarity level of the five core residues (aa 42–46) from 5 matches (SPFSR) to 32 matches (SPASR).

5. DISCUSSION

A fundamental aspect in the study of proteins is the understanding of the effect(s) that mutations have on protein function and/or structure. In immunology, this aspect is crucial. Indeed, the present data demonstrate that the degree of "immunological freedom" is restricted for a peptide sequence. As discussed in detail above, a single aa change can result in a wide spectrum of outcomes in an immunological context, such as reversing inhibition of host innate immune response pathways (54), destroying or improving protein immunogenicity (55, 56), and impairing T-cell activation (57).

Therefore, the essential question remains: how can a single aa residue dramatically alter the immunological properties of a peptide? In this regard, research has annotated many possible explanations. It has been proposed that the changed sequence might contain a determinant that serves to amplify a separate population of lymphocytes (58, 59). Accordingly, Th2 shifts in the immune response and induction of regulatory T cells by altered peptides have been suggested (52). Changes involving anchor residues have been postulated to cause an impact on binding affinity (60, 61). Although of interest, this suggestion does not reconcile the heavy degeneracy of MHC recognition (62–64). With respect to antigenicity, substituted peptides have been suggested to cause perturbation/enhancement of the stereochemical fit between the antigenic peptide and antibody molecules, hence indicating loss/gain of important chemical contributions to antibody interactions (43). However, this mechanism does not explain, for example, why a conservative substitution equates a non-conservative change in destroying a B cell epitope (23).

Here, we analyzed and illustrated data to determine whether a single aa change can modify the immunological information of a peptide module when is accompanied by a loss/gain of sequence similarity. As a matter of fact, a basic concept emerging from our analyses is that a change in the proteomic similarity of a mutated peptide may explain the change in its immunological information. Amino acid changes that transform rare peptide sequences into sequences that repeatedly occur in human proteins "dilute" the immune reaction, which becomes scattered and dispersed among numerous homologous epitopes instead of being concentrated towards one specific target. It appears that a single aa residue substitution can switch on or off the antigenicity/immunogenicity/pathogenicity of proteins and small peptide modules, provided that the aa substitution modifies the sequence similarity level. In essence, as previously advocated by Kanduc (24–28), an increase in the rarity level of a peptide appears to be associated with an increase in its immunological information content. In contrast, becoming a common motif hosted by numerous proteins causes a loss of immunological specificity. The relationship between an aa substitution and a similarity change offers a mathematical mechanism, based on the law of mass action, to explain the shift in immune reactivity. The specificity and strength of peptide-peptide interactions during immune responses do not depend only on the nature of the immune reactants (e.g., antigens, antibodies, T-cell receptors, MHC molecules), but also on the amount of each player in the immune reaction. Logically, a quantitatively/qualitatively high immune response evoked by a rare sequence loses efficacy when fragmented toward

numerous homologous sequences (e.g., antibodies reacting with multiple epitopic homologues).

From a clinical point of view, the fine-tuning of peptide sequence similarity might represent a powerful tool for designing peptides with single aa changes for use in the treatment of specific autoimmune pathologies (e.g., down-regulating the extent of autoimmune attack) and cancer diseases (e.g., up-regulating the specificity of the attack against tumour cells) (24, 32, 34, 35, 65). Likewise, it might be useful in designing peptide-based approaches against infectious microbial organisms, which appear to escape immune surveillance because of their widespread similarity to the human proteins (31-34, 66-74).

6. CONTRIBUTIONS

GL, AAS and DK have equally contributed to data analyses and interpretation. DK conceived the study and wrote the manuscript. All authors discussed and approved the final manuscript.

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