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Design, synthesis and biological evaluation of a new bridged immunosuppressor★✪

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A bridged peptide with the sequence: H-Thr-Pro-Gln-Arg-Gly-Asp-Val-γ-Abu-Asn-Asp-Gln-Glu-Glu-Thr-Thr-Gly-Val-Val-Ser-Thr-Pro-Leu-Ile-Arg-Asn-Gly-OH was designed to mimic the discontinuous epitope of the HLA-DQ molecule that might interact with CD4. The bridged peptide revealed distinct suppressory effect in the humoral immune response. This result supports our suggestion that the 164–172 region of the HLA-DQ molecule may enhance its interactions with coreceptors, possibly with CD4.

HLA-II are cell surface $\alpha\beta$ heterodimers (M_r about 60 000) that play a pivotal role in the immune response by presenting peptides derived from environmental antigens to T-cell receptors. Our previous studies showed that fragments located in the β164–172 loop of HLA-DQ suppress the humoral and cellular immune responses [1, 2] and inhibit some integrins [3]. The fragments contain the RGD sequence, which is known to be important in several proteins in mediating cell adhesion interactions. Our analysis shows that the RGD

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Abbreviations: Abbreviations and symbols are in accordance with recommendations of the European Peptide Society (*J. Peptide Sci.* **5**, 465–471, 1999). DTH, delayed type hypersensitivity; HLA, human leukocyte antigen; PFC, plaque-forming cells.

sequence is located in a loop of the HLA-DQ molecule exposed toward the solvent and, therefore, it may be involved in the interactions with other proteins. We suggested that the loop may serve as a functional epitope on the HLA class II surface for intermolecular binding, and that a possible mechanism of biological action of the synthesized peptides is connected with specific interfering with the adhesion of HLA class II molecules to their coreceptors, e.g. some specific integrins and/or the T-cell coreceptor CD4 [4, 5].

Formerly, Cammarota *et al.* [6] identified the 134–152 fragment of HLA-II molecules as interacting with the coreceptor CD4. Zagury *et al.* [7] showed that synthetic peptides derived from this fragment induce immunosuppression by inhibition of the $CD4^+$ cell immune activation. The fragment is situated on the anti-parallel β-strand located in the vicinity of the β164–172 loop (Fig. 1). Therefore, it is possible that interactions with the loop may additionally enhance the binding between the $CD4^+$-cell receptor and the class II molecules.

To test the possibility that there exist two binding sites on the HLA-II molecule for its coreceptors we designed and synthesized a bridged analog with a linkage between the 164–170 and 137–143 fragments of the molecule. Our molecular modeling study revealed, that a γ-aminobutyric acid bridge (γ-Abu) should be the most efficient in mimicking discontinuous epitopes with motifs on two neighborly located anti-parallel β-strands. The synthetic bridged peptide (1) with the sequence: H-Thr-Pro-Gln-Arg-Gly-Asp-Val-γ-Abu-Asn--Asp-Gln-Glu-Glu-Thr-Thr-Gly-Val-Val-Ser-Thr--Pro-Leu-Ile-Arg-Asn-Gly-OH consists of three

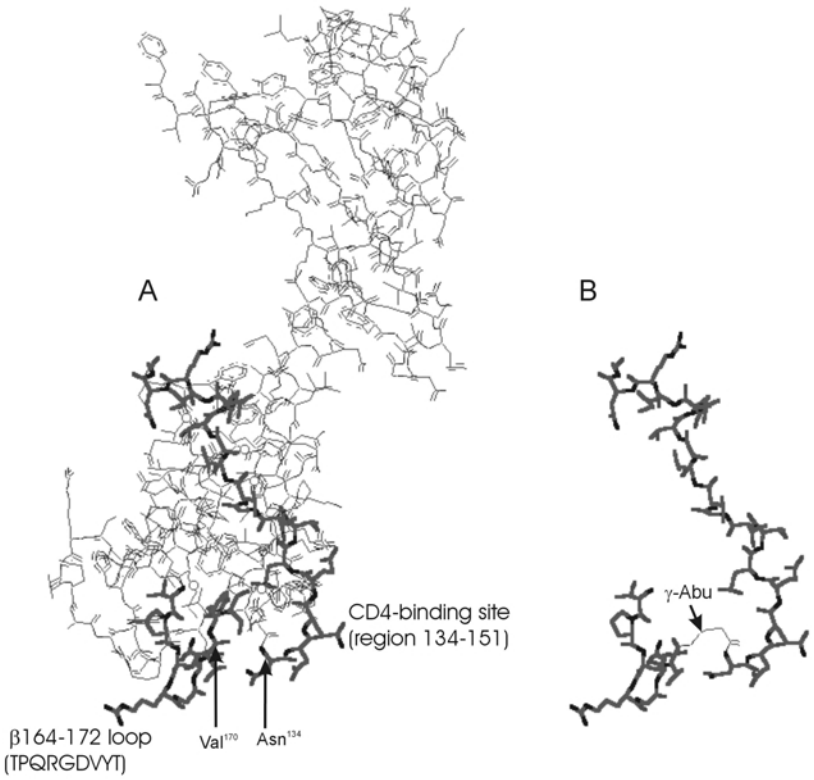

Figure 1. Model of the β2-domain of HLA-DQ (A) and the structure of two-headed peptide 1 (B).

A. Model of the β2-domain of HLA-DQ based on mutational analyses of the HLA-DR crystal structure. The 164–172 and 134–151 fragments are bolded. It is noteworthy that the residues Val^{170} and Asn^{134} (marked with arrows) are in close proximity. B. Structure of two-headed peptide 1 formed by the 164–170 and 134–151 fragments of HLA-DQ connected by a γ-Abu bridge.

segments: (i) the RGD-containing HLA-DQ fragment 164–170, (ii) the linker γ-Abu, and (iii) the fragment that corresponds to the region 137–153 of HLA class II molecules, previously shown to control their interaction with CD4 (Fig. 1B). The peptide was tested for its immunological activities and its potency was compared with its shorter fragments, H-Asn-Asp-Gln-Glu-Glu-Thr-Thr-Gly-Val-Val--Ser-Thr-Pro-Leu-Ile-Arg-Asn-Gly-OH (peptide 2) and H-Ser-Thr-Pro-Leu-Ile-Arg-Asn-Gly-OH (peptide 3). It could be expected that the presence of two contact fragments should increase the chance of interaction of the peptides with CD4 resulting in an increased potency and selectivity. Since CD4 molecules are present mostly on $CD4^+$ lymphocytes, their specific inhibition should lead to suppression of the humoral immune response.

RESULTS AND DISCUSSION

The peptides were synthesized by standard methods. Their structures were confirmed by amino-acid analysis and mass spectrometry. The sequence of hexacosapeptide 1 was determined with electrospray mass spectrometry by collision-induced dissociation (CID) of its positive ion. The observed B_n and Y_n ion series of peptide 1 are given in Fig. 2.

The synthesized fragments of HLA-DQ (1–3) have been investigated for their activity in the regulation of both the cellular and the humoral immune response. The details of all tests have been described previously [8]. The cellular immune response (Fig. 3A) was assayed by determination of the influence of the peptides on the inductive phase of the delayed type hypersensitivity (DTH). Distinct dose dependent immunosuppressive effects of a relatively low magnitude were observed for all of the tested peptides. However, it is clearly visible from this assay that the peptides 1 and 2 exhibit the same potency. Therefore, the introduction of the second binding site to the 134–151 HLA-DQ-fragment does not affect the cellular immune response. The influence of the peptides on the humoral immune response (Fig. 3B) of mice was assayed by counting the

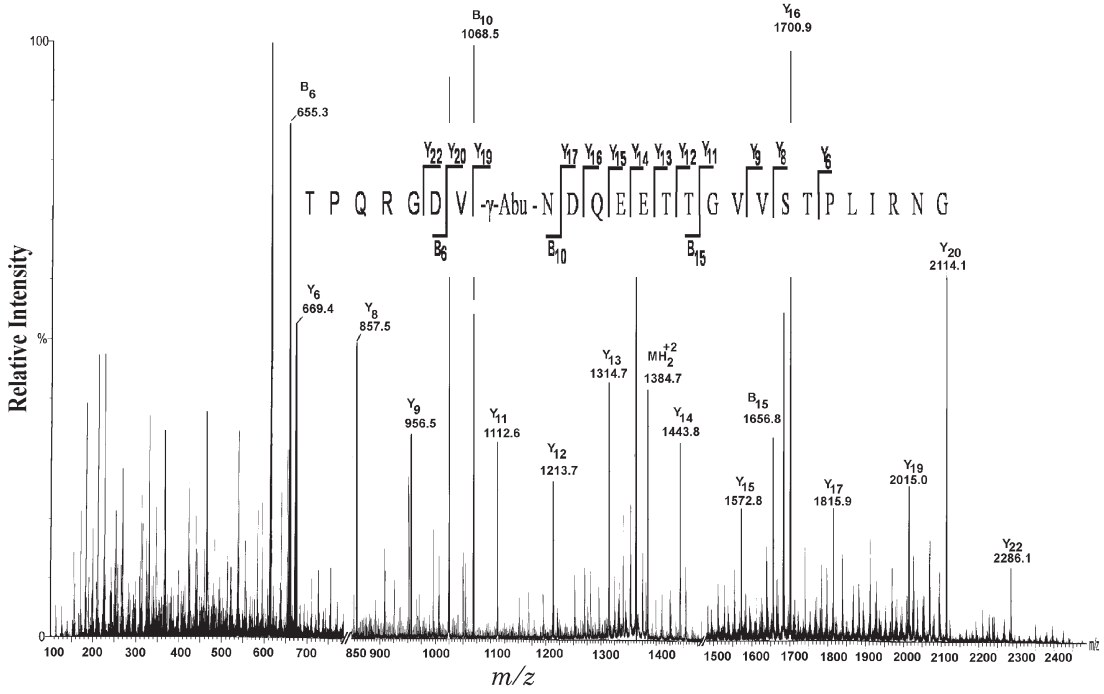

Figure 2. Low-energy CID spectrum of the MH_2^{2+} precursor ion (*m/z* 1384.7) of hexacosapeptide 1 and the observed product ions.

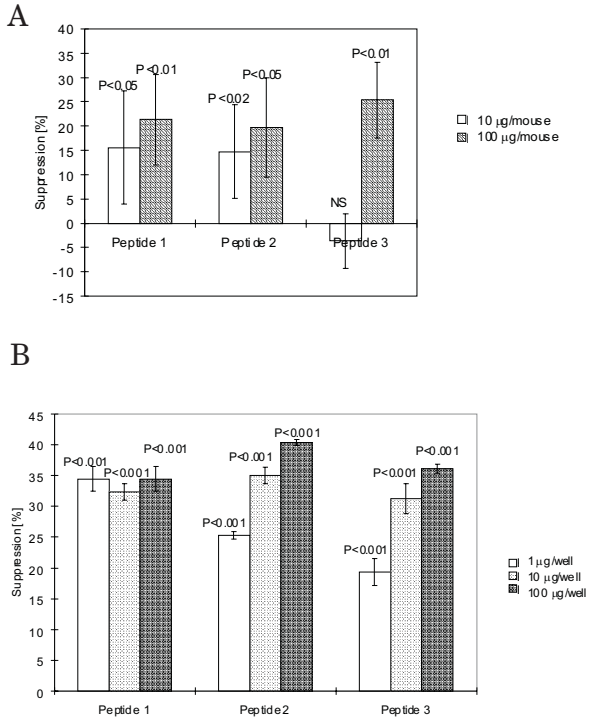

Figure 3. Effect of peptides 1–3 on immunomodulatory activities.

A. Delayed type hypersensitivity (DTH) reaction (inductive phase of foot-pad test) in 129/Iiw mice sensitized with SRBC and treated i.p. with the peptides 24 h after the inductive dose of the antigen. The results are expressed as a mean ±S.E. of 6 mice. B. Direct plaque-forming cells (PFC) numbers in the mouse spleen cell cultures of CBA/Iiw mice immunized with sheep red blood cells (SRBC) and treated i.p. with the peptides. The results are expressed as a mean ±S.E. of 6 wells. To make the comparison of the data easier we present percentage values of the inhibition of the immune responses. The values were calculated according to the equation:

$$\%\ \text{of immunosuppression} = 100\left(1 - \frac{\text{experimental value}}{\text{control}}\right).$$

number of spleen cells that formed plaques with sheep red blood cells (PFC-test). Although all the peptides tested significantly suppress the humoral immune response, the potency of the bridged peptide 1 is not dose-dependent. The dose of 1 μg/well evoked a pronounced inhibitory effect, higher than that of peptides 2 and 3. The increased potency may suggest that our "two-headed" peptide interacts at the same time with two binding sites of CD4.

In summary, we attempted to design a bridged peptide to mimic the discontinuous epitope of the HLA-DQ molecule. The selective immunosuppressory effect in the humoral immune response supports our suggestion that the 164–172 region of the HLA-DQ molecule may enhance its interactions with coreceptors, possibly with CD4.

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REFERENCES

1. Szewczuk, Z., Siemion, I.Z. & Wieczorek, Z. (1996) *Mol. Immunol.* **33**, 903–908.

2. Szewczuk, Z., Wieczorek, Z., Stefanowicz, P., Wilczyński, A. & Siemion, I.Z. (1997) *Arch. Immunol. Exp. Ther.* **45**, 335–341.

3. Szewczuk, Z., Stefanowicz, P., Wilczyński, A., Siemion, I.Z., Cierniewski, C.S., Kralisz, U. & Wieczorek, Z. (1996) *Biopolymers* **40**, 571–583.

4. Szewczuk, Z., Wilczyński, A., Dyba, M., Petry, I, Siemion, I.Z. & Wieczorek, Z. (2000) *Peptides* **21**, 1849–1858.

5. Szewczuk, Z., Buczek, P., Stefanowicz, P., Krajewski, K., Siemion, I.Z. & Wieczorek, Z. (2001) *Acta Biochim. Polon.* **48**, 121–130.

6. Cammarota, G., Scheirle, A.T.B., Doran, D.M., Knorr, R., Bannwarth, W., Guardiola, J. & Sinigaglia, F. (1992) *Nature* **356**, 799–801.

7. Zagury, J.F., Bernard, J., Achour, A., Astgen, A., Lachgar, A., Fall, L., Carelli, C., Issing, W., Mbika, J.P., Picard, O., Carlotti, M., Callebaut, I., Mornon, J.P., Burny, A., Feldman, M., Bizzini, B. & Zagury, D. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7573–7577.

8. Szewczuk, Z., Wilczyński, A., Stefanowicz, P., Fedorowicz, W., Siemion, I.Z. & Wieczorek, Z. (1999) *Mol. Immunol.* **36**, 525–533.