Immunology 1993 **79** 171–173

Prediction of an HLA B8-restricted influenza epitope by motif

A. SUHRBIER, C. SCHMIDT & A. FERNAN
Queensland Institute of Medical Research, Bancroft Centre, Brisbane, Queensland, Australia

Accepted for publication 8 December 1992

SUMMARY

Alignment of previously identified HLA B8-restricted cytotoxic T-cell (CTL) epitopes revealed a common motif: x, x, **K/R**, x, **R/K**, x, **W/Y**, x, **I/L**. Of five motif-conforming peptides tested, ELRSRYWAI was found to represent a new epitope from influenza nucleoprotein (NP). High concentrations of some peptides gave cross-reactive stimulation illustrating the requirement to confirm epitope assignment with recombinant vaccinias and highlighting a potential problem of peptide-based CTL vaccines.

The realization that major histocompatibility complex (MHC) displays allele-specific binding motifs[1] and that most HLA class I-restricted peptides appear to be eight to 10, usually nine, amino acids long,[1,2] promises to improve vastly the speed and reliability of cytotoxic T-cell (CTL) epitope prediction.[3]

We have recently reported a minimal nine amino acid CTL epitope from A-type Epstein–Barr virus (EBV) located in the EBV nuclear antigen 3 (EBNA 3).[4] This epitope, FLRGRAYGL, was restricted to HLA B8 and most HLA B8, EBV seropositive individuals have CTL, which recognize this epitope (S. Burrows and C. Schmidt, unpublished observation). Such CTL kill lymphoblastoid cell lines (LCL) transformed with A-type virus but not B-type virus, because of two substitutions in the equivalent region of the B-type EBNA 3.[5] (The A-type B95.8 transformants are also not killed[4,5] and were not used in this study.)

Alignment of FL**R**G**R**AYG**L** with other known HLA B8-restricted epitopes[6] revealed a sequence motif (shown in bold; Table 1), which was used to search the EMBL protein sequence data base. A sequence conforming to this motif, EL**R**SR**Y**WA**I**, was found to represent a new epitope from influenza nucleoprotein (NP). Bulk CTL cultures from three HLA B8 donors stimulated with ELRSRYWAI peptide recognized influenza and vacc.NP (vaccinia coding for NP) infected target cells through HLA B8 (Fig. 1a).

Several other conforming peptides were also tested: AD**R**P**R**AW**R**L, PARSL**R**EWEL (EBV, EBNA 5), QR**R**YR**R**IYPL (EBV, EBNA 6), and IV**K**QR**R**WK**L** (EBV, EBNA 4). The first three did not generate, in two HLA B8 EBV seropositive donors, CTL cultures capable of recognizing autologous HLA B8 A-type LCL (only one donor shown; Fig. 1b). IVKQRRWKL did stimulate an A-type response, but such cultures did not kill vacc.EBNA 4-infected LCL but recognized vacc.EBNA 3-infected and FLRGRAYGL-sensitized LCL. Curiously, ELRSRYWAI also stimulated the same response (Fig. 1b). IVKQRRWKL and ELRSRYWAI (used at 50 μM) were thus capable of stimulating FLRGRAYGL-specific CTL. This conclusion was supported by the observation that an FLRGRAYGL-specific CTL clone, LC13,[4] was capable of lysing target cells sensitized with IVKQRRWKL or ELRSRYWAI, although recognition required a much higher concentration of peptide (Fig. 2). Neither influenza- nor vacc.NP-infected HLA B8 target cells were recognized by LC13 or stimulated an FLRGRAYGL-specific response (data not shown), illustrating how high exogenous peptide concentrations can override the specificity imposed by physiological levels of peptide presentation. Definition of peptide epitopes by use of peptides alone could thus generate misleading results; if FLRGRAYGL was not described, the absence of vacc.EBNA 4 data might lead to the conclusion that IVKQRRKWKL was an HLA B8-restricted epitope from EBV. Such partial cross-reaction between peptides might need to be considered if synthetic peptides are to be used as CTL-based vaccines. The use

Table 1. HLA B8-binding motif. The residues conforming to the ('full house' match) motif (x, x, **R/K**, x, **R/K**, x, **Y/W**, x, **I/L**) are written in bold. Suggested minimal nine amino acid sequences are underlined

	Source
Known HLA B8-restricted epitopes	
FL**R**G**R**AYG**L**	EBV[4]
IETVPVKLKPGMDGP**K**V**K**O**W**PLTEE	HIV[6]
NPPIPVGEIY**K**R**W**II	HIV[6]
YL**K**DQQL-**L**	HIV[6]
LRPGG**KK**K**Y**KLKHIV	HIV[6]
VQNANPDC**K**TILKA**L**	HIV[6]
Predicted epitope	
EL**R**SRY**W**A**I**	Influenza

Correspondence: Dr A. Suhrbier, Queensland Institute of Medical Research, 300 Herston Road, Herston, Brisbane, Queensland, Australia 4029.

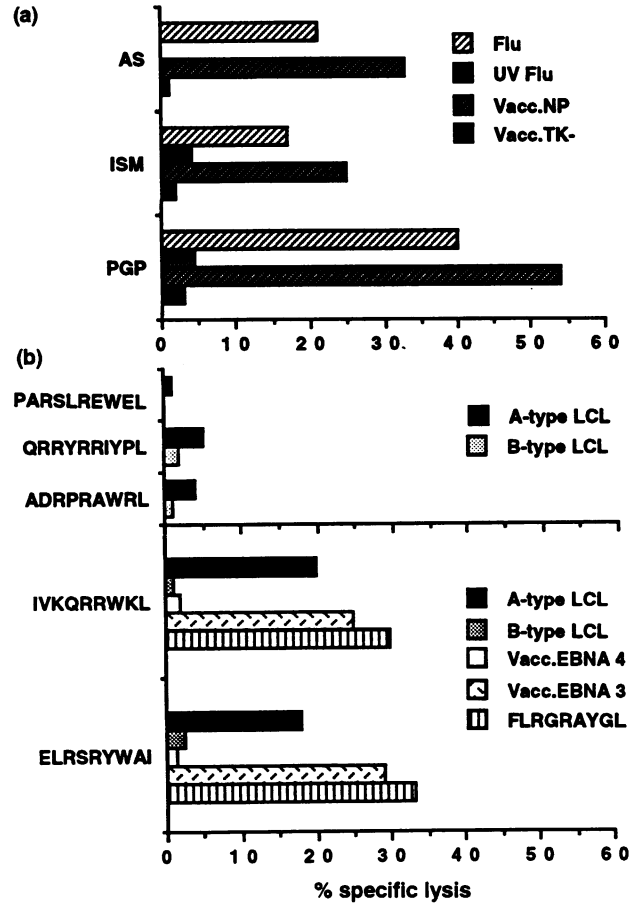

Figure 1. (a) ELRSRYWAI stimulates CTL specific for an influenza nuclear protein. 2×10^6 peripheral blood mononuclear cells (PBMC) from donors AS (HLA A1, Bw6, 8), ISM (A1, 24, B51, 8) and PGP (A1, 24, B14, 8) were stimulated with 50 μM ELRSRYWAI and maintained for 7 days in 10% human serum, RPMI-1640 followed by 3 days in the same medium containing 10 U/ml recombinant interleukin-2 (IL-2). Target cells were LCL from donor WH (A3, 30/31, B14, 8) infected with live (Flu) or UV-inactivated influenza (UV Flu) (strain A/PR/8/34—kind gift from A. W. Hampson, CSL, Australia; 10 infectious particles/cell) or vaccinia coding for vacc.NP[13] or control TK vaccinia. E:T ratio 20:1. (b) PBMC from donor AS were stimulated with the indicated peptide as above. Targets were A and B-type LCL from donor PGP, or PGP B-type LCL infected with vacc.EBNA 3 or 4,[14] or B-type LCL sensitized with 50 μM FLRGRAYGL.[4]

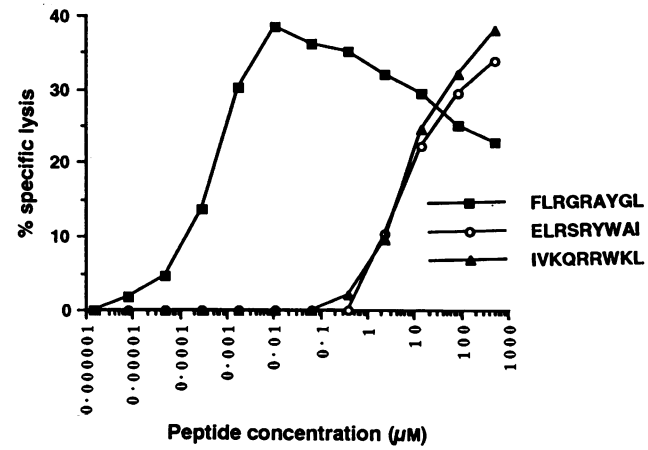

Figure 2. CTL clone LC13 specific for FLRGRAYGL also recognizes ELRSRYWAI and IVKQRRWKL. Target cells were autologous B-type LCL.[4] E:T ratio 2:1. (All the peptides in the study had free amino and carboxy termini.)

of ELRSRYWAI, at concentrations above 1 μM, as an influenza vaccine, might stimulate pre-existing FLRGRAYGL-specific CTL, without priming the small number of influenza-specific precursor CTL; a CTL version of original antigenic sin.[7]

As a large proportion of the peptide/MHC binding energy comes from interactions with the peptide backbone,[2] it is unclear how many of the six proposed HLA-binding pockets[8] need to be occupied or what constraints are imposed by their specificity and the conformation of the peptide.[2] Such information may refine motifs but the 'sloppy nature'[2] of peptide side chain/MHC interactions may limit the precision of CTL epitope prediction by motif. Peptide side chains may, for instance, be selected by their ability not to get in the way of, rather than contribute to, MHC peptide interaction. Clearly other restrictions will also determine whether a sequence will be presented as an epitope: processing;[9] peptide transporter specificity;[10] protein/pathogen biology;[11] competition.[12] Information concerning these processes are likely to be required in conjunction with HLA-binding motifs in order to accurately predict T-cell epitopes.

ACKNOWLEDGMENTS

This work was supported by the Wellcome Trust, U.K.; the National Health and Medical Research Council, Australia; the Tropical Health Program, Australia; Queensland Cancer Fund, Australia and The National Cancer Institute, USA (NIH CA 52250.01).

REFERENCES

1. Strauss H.J. (1991) Peptides feeling groovy. *Curr. Biol.* **1,** 328.
2. Barinaga M. (1992) Getting some "Backbone": how MHC binds peptide. *Science*, **257,** 880.
3. Pamer E.G., Harty J.G. & Bevan M.J. (1991) Precise prediction of a dominant class I MHC-restricted epitope of *Listeria monocytogenes*. *Nature*, **253,** 852.
4. Burrows S.R., Rodda S.R., Suhrbier A., Geyson H.M. & Moss D.J. (1992) The specificity of recognition of a cytotoxic T lymphocyte epitope. *Eur. J. Immunol.* **22,** 191.
5. Apolloni A., Moss D.J., Stumm R., Burrows S.R., Suhrbier A., Misko I.S. & Scully T.B. (1991) Sequence variation of a cytotoxic T cell epitope in different isolates of Epstein–Barr virus. *Eur. J. Immunol.* **22,** 183.
6. Johnson R.P., Trocha A., Buchanan T.M. & Walker B.D. (1992) Identification of overlapping HLA Class I-restricted cytotoxic T cell epitopes in a conserved region of the Human Immunodeficiency Virus Type 1 envelope glycoprotein: definition of minimum epitopes and analysis of the effects of sequence variation. *J. exp. Med.* **175,** 961.
7. Benjamini E., Andria M.L., Estin C.D., Notron F.L. & Leung C.Y. (1988) Studies on the clonality of the response to an epitope of a protein antigen. Randomness of activation of epitope-recognizing clones and the development of clonal dominance. *J. Immunol.* **141,** 55.
8. Murray N. & McMichael A. (1992) Antigen presentation in virus infection. *Curr. Opin. Immunol.* **4,** 401.
9. Eisenlohr L.C., Yewdell J.W. & Bennick J.R. (1992) Flanking sequences influence the presentation of an endogenously synthesized peptide to cytotoxic T lymphocytes. *J. exp. Med.* **175,** 481.

10. MONACO J.J. (1992) A molecular model of MHC class-I-restricted antigen processing. *Immunol. Today*, **13,** 173.
11. LONG E.O. & JACOBSON S. (1989) Pathways of viral antigen processing and presentation to CTL: defined by mode of virus entry? *Immunol. Today*, **10,** 45.
12. ADORINI L. & NAGY Z.A. (1990) Peptide competition for antigen presentation. *Immunol. Today*, **11,** 21.
13. MCMICHAEL A., MICHIE C.A., GOTCH F.M., SMITH G.L. & MOSS B. (1986) Recognition of influenza A virus nucleoprotein by human cytotoxic T lymphocytes. *J. exp. Med.* **67,** 719.
14. KHANNA R., BURROWS S.R., KURILLA M.G., JACOB C.A. MISKO I.S., SCULLY T.B., KIEFF E. & MOSS D.J. (1992) Localisation of Epstein–Barr virus cytotoxic T cell epitopes using recombinant vaccinia: implications for vaccine development. *J. exp. Med.* **176,** 169.