(20 mg ml^{-1} HLA A2/pol peptide complex in 10 mM Tris-HCl, 10 mM NaCl, pH 8.0, mixed in 2:1 stoichiometry with 10 mg ml^{-1} CD8αα in the same buffer) plus 1 μl of reservoir solution (12% PEG 20000, 100 mM MES pH 6.5). The presence of both HLA-A2 and CD8αα molecules in the crystals was demonstrated by polyacrylamide gel electrophoresis. Crystals were harvested into a modified reservoir solution containing 15% PEG 20000, 50 mM MES, pH 6.5. For cryogenic data collection, crystals were transferred stepwise to harvest buffer supplemented with progressively higher concentrations of glycerol up to a final concentration of 20%, and flash-frozen by plunging into liquid propane. A data set was collected in-house (with crystal maintained at 100 K in a Cryostream; Oxford Cryosystems) using a 30-cm diameter MAR-Research detector mounted on a Rigaku RU200 generator ($\lambda = 1.54$ Å) fitted with Yale mirror optics. Crystals belong to the space group $P2_12_12_1$, with unit cell dimensions $a = 70.9$ Å, $b = 89.6$ Å, $c = 116.0$ Å, and contain one HLA-A2 and one CD8αα dimer per asymmetric unit. The data were auto-indexed and integrated with the program DENZO[20] followed by scaling using the program SCALEPACK[20] to yield a data set 96.5% complete at 2.65 Å ($R_{merge} = 9.5\%$; Table 1).

Structure determination. The structure was determined by molecular replacement using the program AMoRe[21]. Search probes consisted of the original HLA-A2 model, which does not contain a peptide antigen (refined at 2.6 Å; Protein Data Bank accession code 3HLA), and the CD8αα homodimer (refined at 2.6 Å, Protein Data Bank accession code 1CD8). Unambiguous solutions were found in the cross-rotation and translation functions for both molecules which when combined resulted in an R_{cryst} of 43.4% ($R_{free} = 45.3\%$) for data between 10.0 to 3.5 Å. After four-domain rigid-body refinement (α1α2, α3, β_2m and CD8αα) with X-PLOR, the R_{cryst} dropped to 39.4% ($R_{free} = 40.2\%$) for data between 15.0 and 3.0 Å. $2F_o$-F_c and F_o-F_c electron density maps calculated on the basis of these phases showed clear density for the peptide. These maps also presented poor density for several regions, including some of the CDR-like loops, which were removed from the model and gradually rebuilt during the course of the refinement.

Refinement. Refinement was carried out with standard protocols in program X-PLOR[22], maintaining tight non-crystallographic symmetry restraints between the two subunits of CD8αα, and was alternated with manual rebuilding in the interactive graphics program O[23]. A bulk solvent correction allowed all measured data from 15.0 to 2.65 Å to be used. In the final stages of the refinement, ordered water molecules were added to the model in the interface regions. Refinement statistics are given in Table 1. The refined atomic model of the complex comprises residues 1–114 of both CD8α subunits, residues 1–276 of the HLA-A2 heavy chain, residues 2–99 of β_2m, the 9-residue peptide and 108 ordered water molecules. Of the non-glycine residues, none lie in an energetically disallowed region of the Ramachandran plot. For residues 27–30 in CD8α-2 the quality of the electron density is poor but sufficient to define unambiguously the course of the polypeptide mainchain for this apparently flexible loop region. No electron density is observed for residues 115–120, which are presumed to form part of an extended stalk in the intact extracellular region of CD8 (ref. 6).

Received 13 March; accepted 18 April 1997.

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Acknowledgements. We are grateful to the late Alan Williams for discussions that inspired this work. We thank R. Bryan, K. Measures and R. Esnouf for computing facilities and programs; K. Harlos for assistance with X-ray data collection, S. Lee for help in the preparation of figures; Z. Rao for assistance in crystallization trials; D. Wiley and D. Garboczi for pre-release coordinates of the TCR/Tax/HLA-A2 complex; and C. O'Callahan, V. Cerundolo, D. Garboczi, G. Harcourt and B. Willcox for help and advice. This work was funded by the MRC. The Oxford Centre for Molecular Sciences is supported by the BBSRC, EPSRC and MRC. J.T. was supported by an EMBO fellowship, E.Y.J. by the Royal Society, and D.I.S. and A.J.M. by the MRC.

Correspondence and requests for materials should be addressed to J.I.B. (e-mail: john.bell@ndm.ox.ac.uk) or E.Y.J. (e-mail: yvon@biop.ox.ac.uk). Atomic coordinates for the CD8αα/HLA-A2/peptide complex have been deposited with the Protein Data Bank (Brookhaven National Laboratory), and are available prerelease from yvon@biop.ox.ac.uk and jose@biop.ox.ac.uk.

correction

CD8 enhances formation of stable T-cell receptor/MHC class I molecule complexes

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Nature **384,** 577–581 (1996)

No bands were evident in the first three lanes of the gel presented in Fig. 4. The corrected figure is shown here. □