The Association of \( p56^{\text{ck}} \) with the CD4/CD8 Antigens: Implications for T-Cell Function

C.E. Rudd and S.F. Schlossman

INTRODUCTION

The CD4 and CD8 antigens define discrete T-cell subsets involved in the restricted recognition of MHC class II and I antigens, respectively. The CD4 antigen also appears to serve as a receptor for the human immunodeficiency virus (HIV), the causative agent of AIDS. Both sets of antigens are members of the immunoglobulin superfamily and appear to synergize with the T-cell receptor complex (\( T\text{cR}/CD3 \)) in the initiation of T-cell proliferation (Eichmann et al. 1986, Anderson et al. 1987). An important question has been to understand the underlying molecular basis of CD4 and CD8 function. In this paper, we briefly review data demonstrating a physical interaction between the CD4 or CD8 antigens and the protein-tyrosine kinase \( p56^{\text{ck}} \) and outline its potential importance in the regulation of T-cell growth.

\( p56^{\text{ck}} \) Is Associated With the CD4 and CD8 Antigens

The CD4 and CD8 antigens have been found to be physically associated with the catalytically active form of the protein-tyrosine kinase \( p56^{\text{ck}} \) from T lymphocytes (Rudd et al., 1988; Barber et al., 1989). As seen in Figure 1, immunoprecipitates of CD4 and CD8 were found capable of transferring the \( [\gamma] \) phosphate of \( [\gamma-\text{ATP}] \) to a co-precipitated substrate resulting in the labelling of a band at 55-60KD (lane 2 and 3, respectively). Further, phosphoamino acid analysis indicated labelling at a tyrosine residues, a extremely rare form of protein modification, accounting for only 0.01-0.02 percent of phosphorylation in the cell. Neither CD4 nor CD8 possess definable protein-tyrosine kinase domains. It was therefore likely that a protein tyrosine kinase was being co-purified with both CD4 and CD8. One candidate was the protein-tyrosine kinase \( (p56^{\text{ck}}) \) with a Mr of 56-62KD which has been reported to be expressed specifically in human T cells. To assess whether \( p56^{\text{ck}} \) was associated with the CD8 receptor, an antisera to the carboxy terminus of \( p56^{\text{ck}} \) was shown to specifically re-precipitate the 55-60Kd band after denaturation of the anti-CD8 precipitate in SDS (lane 10). Labelling at tyrosine was confirmed by re-precipitation with an anti-phosphotyrosine antibody (lane 11) and by phosphoamino acid analysis (Fig. 1B). Identical results were obtained in re-precipitation studies from anti-CD4 precipitates (Rudd et al. 1988). Two dimensional NEPHGE/SDS-PAGE further showed that the kinase associated with the CD8 antigen had the same Mr and charge (approximate pI of 4.8 to 5.8) as that associated with the CD4 antigen (Fig. 1C, middle panel and lower panel, respectively), and with that recognised directly by the anti-\( p56^{\text{ck}} \) antiserum from detergent lysates (Fig. 1C, upper panel). Collectively, these data revealed that similar if not identical forms of catalytically active p56^{ck} associated with the CD4 and CD8 antigens.
active p56<sub>1ck</sub> are associated with the CD4 and CD8 antigens from T cells. The association is specific in that numerous other T-cell antigens have failed to co-precipitate kinase activity (Rudd et al., 1988).

**Figure 1**  
(A) Phosphotransferase activity of immunoprecipitates derived from REX (lanes 1-4) and MOLT 4 cells (lanes 5-8). (1,5) rabbit anti-mouse antibody; (2,6) anti-CD4 antibody; (3,7) anti-CD8 antibody; (4,8) anti-p56<sub>1ck</sub> antiserum; (9) anti-CD8 reprecipitated with the W6/32 antibody; (10) anti-CD8 reprecipitated with the anti-p56<sub>1ck</sub> serum; (11) anti-CD8 reprecipitated with an anti-phosphotyrosine antiserum. (B) Phosphoamino acid analysis of the 55-60KDa band. (C) Two Dimensional NEPHGE/SDS-PAGE of p56<sub>1ck</sub> Labelled in the Phosphotransferase Assay. Upper panel: anti-p56<sub>1ck</sub> antiserum; middle panel: anti-CD8 antibody; lower panel: anti-CD4 antibody.

The CD4 and CD8 antigens are known to synergise with the Ti(TcR)/Ti complex in the stimulation of T cells. Increasing the physical proximity of the CD4 and CD8 antigens with the Ti (TcR)/CD3 complex potentiates the activation of T-cells (Emmerich et al., 1986; Anderson et al., 1987). An important question is whether this interaction is mediated by CD4 and CD8 associated p56<sub>1ck</sub>. Importantly, as seen in Figure 2B, the CD4 and CD8:p56<sub>1ck</sub> complexes readily phosphorylated members of the CD3 complex when co-purified and labelled in a phosphotransferase assay. The anti-CD3 immunoprecipitate alone did not co-precipitate kinase activity, while the anti-CD8 antibody precipitated the labelled p56<sub>1ck</sub> at 55-60KD (lanes 1 vs 3, respectively). However, the addition of [γ<sup>32</sup>P]-ATP to the CD8:p56<sub>1ck</sub> complex in the presence of CD3 resulted in the dramatic labelling of additional bands at 20 to 26KD.