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TYROSINE PHOSPHORYLATION IN HUMAN T LYMPHOMA CELLS

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A high level of tyrosine protein kinase (TPK) has been recently detected in the murine lymphoma LSTRA. The main substrate for tyrosine phosphorylation in this cell line is a Mr 55 000 protein associated with the insoluble matrix of the cell. These findings prompted the search for TPK activities in human lymphoïd cells. Three human T lymphoma cell lines (i.e. Molt. 4, JM, and Ke 37) and control lymphocytes were examined. After in vitro phosphorylation of detergent insoluble extracts from human T lymphoma cells, 2 major phosphotyrosine containing proteins with Mr 55 000 and 35 000 can be detected in all three T lymphoma lines, whereas an additonal specie with Mr 78 000 is present only in the Ke 37 cell line. Similar size proteins are weakly phosphorylated in normal lymphocytes. Tyrosine phosphorylation in these proteins proceeds actively at 0° C, and is dramatically stimulated by Mn^{++} ions. Partial proteolysis mappings of the Mr 55 000 phosphoproteins from murine and human lymphomas revealed a strong homology among these molecules. The function of this protein in transformed lymphocytes is discussed.

A new class of protein kinases has been discovered in recent years, which possess the unique capacity to phosporylate tyrosine residues. Various lines of evidence suggest that these enzymes are involved in the control of cell proliferation : i) the protein products of several retroviral transforming genes exhibit tyrosine protein kinase (TPK) activities (for a review see ref. 1) and ii) certain physiological growth factors such as epidermal growth factor (EGF), platelet derived

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growth factor (PDGF) and insulin have recently been shown to stimulate tyrosine phosphorylation in their receptors (2,3,4). These enzymes which are mainly localized in the plasma membrane and/or associated with cytoskeletal structures, can be recovered in the detergent insoluble material (DIM) of the cells (5,6).

Recently, a Mr 55 000 phosphoprotein (p55) containing phosphotyrosine has been detected in the murine lymphoma cell line LSTRA. This phosphoprotein found within the DIM of LSTRA cells, after both in vitro and in vivo phosphorylation (7,8), reflects the presence of a very active TPK in this cell line. This finding prompted the search for similar enzymatic activities in human T lymphoma cell lines. 2 to 3 phosphotyrosine containing proteins can be demonstrated after in vitro phosphorylation of the DIM in three T cell lines examined. In all cases, a Mr 55 000 phosphoprotein containing phosphotyrosine is detected, which is closely homologous to murine p55. The possible function of this protein and its role with respect to proliferation of lymphoïd cells are discussed.

MATERIAL AND METHODS

The human cell lines Ke 37, Molt 4 and JM are three T lymphoma cell lines originally isolated from human patients with acute lymphoblastic leukemia ; they were cultured in RPMI 1640 medium supplemented with 10 % foetal calf serum (FCS). LSTRA lymphoïd tumor line was originally isolated as a transplantable tumor following inoculation of BALB/c newborn mice with Moloney murine leukemia virus (Mo-MuLV) and grown in culture in the same conditions as human T lymphoma cells. Normal lymphocytes were isolated from heparinized blood of normal donors, through Ficoll-Hypaque gradients.

Preparation and phosphorylation of DIM were performed according to described procedures (8). Briefly, washed cells were lysed in DIM buffer (10 mM pipes pH 6.8, 100 mM KCl, 300 mM Sucrose, 1 mM PMSF, 100 Kiu/ml Aprotinine) containing 1 % triton X 100. DIM was washed and resuspended in the same buffer, and then phosphorylated in the presence of $MnCl_2$ or $MgCl_2$ 5 mM and $\gamma[^{32}P]$ ATP (150µCi/ml).

Phosphorylated extracts were resolved on SDS-polyacrylamide gradient gels (7,5 - 15 %) and phosphoproteins were detected by autoradiography. Detection of phosphotyrosine was achieved by taking advantage of the relative alkali-stability of the ester linkage of this compound. Gels were soaked in 2N NaOH for 1 h at 55° C with gentle shaking, dried and autoradiographed. The presence of phosphotyrosine in the alkali resistant phosphoproteins was confirmed by phosphoamino-acid analysis on cellulose acetate plates (9).

Partial proteolysis mappings were performed according to Cleveland et al. (10) with papaïne (Sigma) and staphylococcus aureus V_8 protease (Miles).

RESULTS

Phosphorylation patterns of the DIM from three human T lymphoma cells (i.e. Molt4, JM, and Ke 37) are presented in figure 1, along with those from LSTRA cells and normal human lymphocytes. Human T lymphoma extracts exhibit strongly labelled phosphoproteins resistant to alkali treatment of the gel ; these include two major proteins with Mr 55 000 and 35 000 present in all cases and an additionnal broad band with Mr 78 000 specific to the Ke 37 cell line (fig. 1B). Weakly phosphorylated proteins with similar sizes can also be detected in normal cell extracts. In agreement with our previous report (8), the DIM from LSTRA cells contains a heavily labelled phosphoprotein of Mr 55 000 which is resistant to alkali hydrolysis. Phosphoaminoacid analysis of the alkali resistant phosphoproteins detected in human lymphoma cell extracts disclosed exclusively phosphotyrosine in all cases (fig. 2). Similarly, phosphotyrosine was found in alkali stable phosphoproteins from normal lymphocytes (S. Fischer et al., submitted for publication) and in p55 from LSTRA (8).

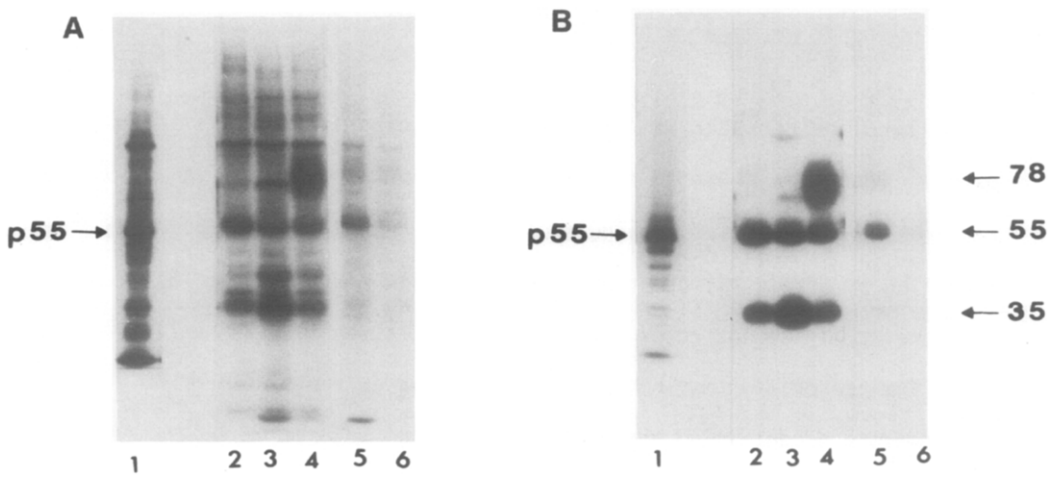

Figure 1 : Phosphorylation patterns of the detergent insoluble material (DIM) from murine and human lymphoma cells.
DIM were phosphorylated with γ ^{32}P ATP (150 µCi/ml), for 5 minutes at 0°C and analysed on SDS polyacrylamide gels. The gels were autoradiographed prior to alkali treatment (panel A) and after treatment with NaOH (panel B).
Phosphorylated DIM from : 1 : LSTRA 2 : Molt 4 3 : JM 4 : Ke 37 5 and 6 : normal human lymphocytes.

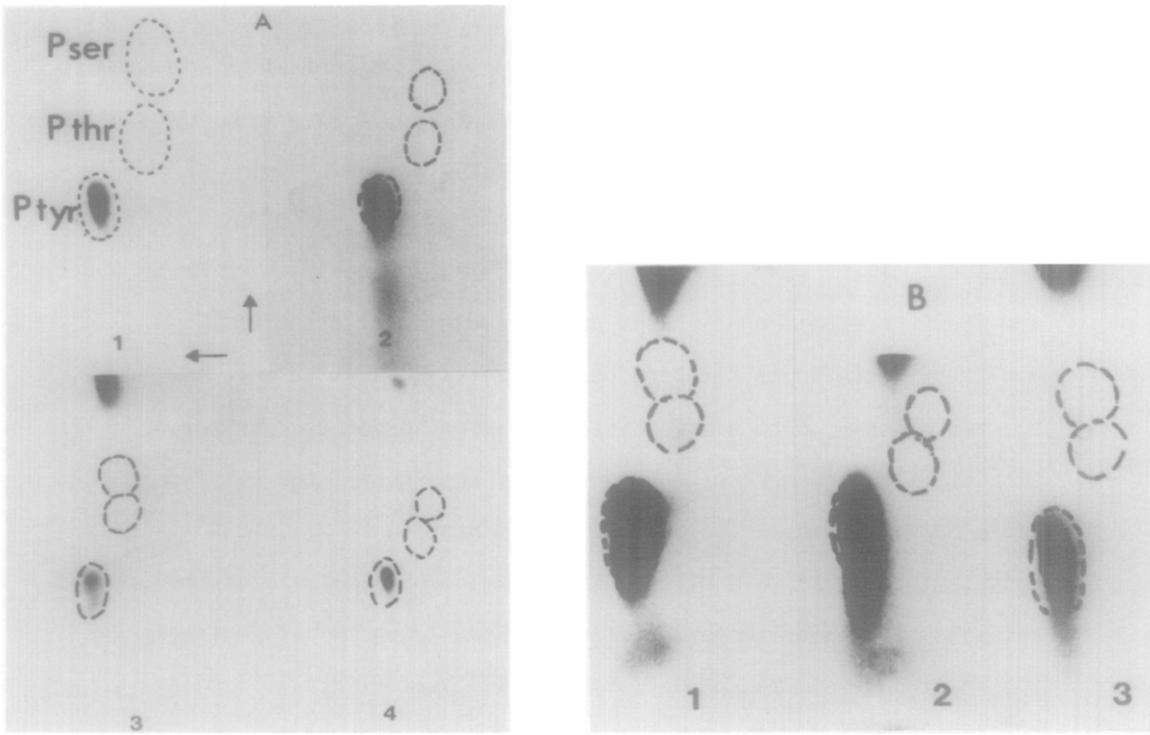

Figure 2 : Analysis of phophoaminoacids in alkali-stable phosphoproteins detected in human T lymphoma cells.
Gel slices containing alkali resistant phosphoproteins with Mr 35 000 (p35), 55 000 (p55), and 78 000 (p78) were submitted to acid hydrolysis ; the resulting aminoacid mixtures were resolved by electrophoresis-chromatography on cellulose acetate plates as described (8,9).
Panel A : 1 : p55 from JM 2 : p35 from JM
3 : p55 from Molt 4 4 : p35 from Molt 4
Panel B : 1 : p78 from Ke 37 2 : p55 from Ke 37
3 : p35 from Ke 37

Tyrosine phosphorylation in these proteins is strongly stimulated by Mn^{++} ions ; as shown in figure 3, substitution of $MgCl_2$ by $MnCl_2$ in the phosphorylation mixture results in a 10 to 20 fold increase in the labelling of alkali resistant phosphoproteins. A similar effect has been previously demonstrated for p55 phosphorylation in LSTRA cells (7).

The presence in murine and human lymphoma extracts of a Mr 55 000 phosphoprotein containing phosphotyrosine led us to examine a possible relatedness between these phosphoproteins. Partial proteolysis mappings of the Mr 55 000 phosphoproteins from murine and human origin were compared. As illustrated in figure 4, the phosphopeptide patterns obtained after partial proteolysis of the various Mr 55 000 phosphoproteins

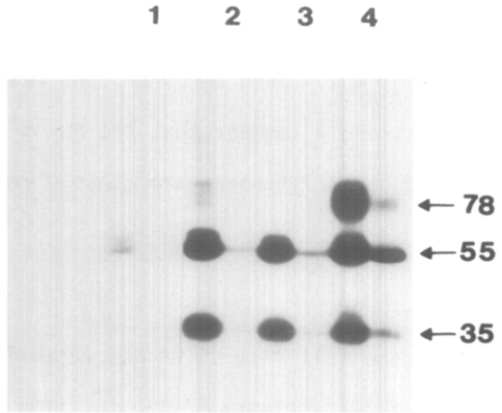

Figure 3 : Effect of Mn^{++} ions on tyrosine phosphorylation in DIM from human T lymphoma cells and control lymphocytes.
1 : normal lymphocytes
2 : JM
3 : Molt 4
4 : Ke 37
Each sample was phosphorylated in the presence of either 5 mM $MnCl_2$ (left lane) or 5 mM $MgCl_2$ (right lane).

with papaïne are strikingly similar if not identical. Moreover, despite some minor differences (i.e. a Mr 30 000 phosphopeptide detected only into two human lymphomas and an extra band of low Mr in Ke 37 cell line),

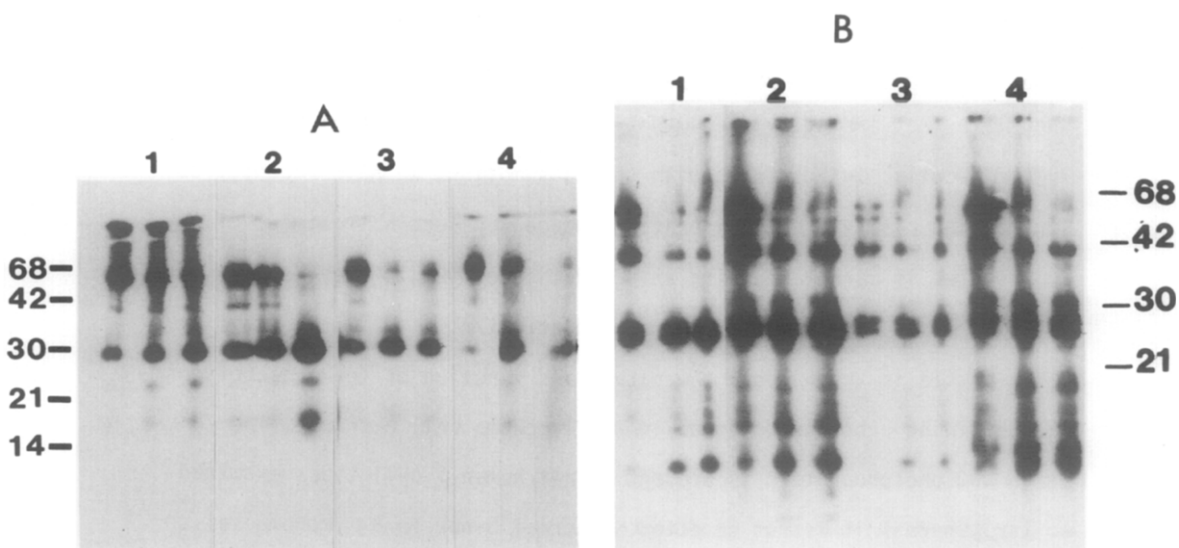

Figure 4 : Partial proteolysis mappings of the Mr 55 000 phosphoproteins (p55) from murine and human lymphomas.
Gel slices containing p55 were excised and applied to a second gel together with proteases.
Panel A : digestion with papaïne
Panel B : digestion with staphylococcus aureus V_8 protease.
p 55 from : 1 : LSTRA 2 : Ke 37 3 : JM 4 : Molt 4

the patterns obtained with staph. aureus V_8 protease also exhibit remarkable similarities (fig. 4B). Taken together, the results of partial proteolysis mappings strongly suggest that the Mr 55 000 phosphoproteins detected in murine and human lymphomas are closely related if not identical molecules.

DISCUSSION

The murine lymphoma cell line LSTRA has recently been shown to contain elevated levels of TPK activity (7,8). In vitro, a major endogenous substrate for the tyrosine protein kinase activity in the detergent insoluble material of these cells is a protein of Mr 55 000 (p55). These findings prompted us to examine TPK activities in human lymphoïd cells including T lymphoma cells and control lymphocytes.

As judged from in vitro phosphorylation experiments, all three lymphoma cell lines examined contain appreciable levels of TPK activities. These activities are present within the DIM, suggesting that enzymes are associated with cytoskeleton and/or membrane. They are active at 0° C and dramatically stimulated by Mn^{++} ions. These properties are shared by TPK activities from the murine lymphoma LSTRA and by protein kinases encoded by retroviral transforming genes. It is thus tempting to speculate that TPK activities might play a role in the proliferation of lymphoïd cells. In agreement with this assumption, we have recently observed that blast-like proliferation induced by lectin treatment of T lymphocytes is correlated with an activation of TPK in these cells (S. Fischer et al., submitted for publication).

Little is known about the nature and function of the phosphotyrosine containing proteins detected in the lymphoma cell extracts. The Mr 35 000 phosphoprotein is present in all human T cell types examined so far, whereas it is not detected in normal B nor in B lymphoma cells (our unpublished observations) ; it might therefore be specific of the T differentiation stage. As for the Mr 78 000 phosphoprotein found in th Ke 37 cell line, we have no explanation for its presence in this unique cell line. The most striking result of this work arises from the

comparison of the Mr 55 000 phosphoproteins present in the various murine and human lymphoma lines examined. As judged from the phosphopeptide patterns obtained by partial proteolysis mapping, it seems likely that closely related if not identical molecular species are present in the four different cell lines. The weakly phosphorylated protein of Mr 55000, detected in control lymphocytes seems to be also structuraly related to the p55 present in lymphoma extracts (data not shown). Assuming that p55 is involved in the control of T cell proliferation, the main question is that of its function. In this connection one can speculate whether a TPK activity is associated with p55. According to previous studies of virally encoded protein kinases, all of these enzymes are phosphorylatable on specific tyrosine residues presumably by autophosphorylation reactions. Thus it is conceivable that p55 which is, at least in the case of LSTRA cells, the main phosphotyrosine containing protein, bears itself the TPK activity. In view of the size of p55, the possible relatedness between this protein and the 60 000 daltons protein kinase encoded by RSV ($pp60^{src}$) has been investigated ; this hypothesis has been clearly ruled out by comparative partial proteolysis mappings of p55 and $pp60^{src}$ since they disclosed different phosphopeptide patterns (11 and our unpublished observations). Although we cannot eliminate the hypothesis that p55 might be the autophosphorylated kinase, it could, alternatively, be a substrate for an unidentified TPK. To our knowledge, no substrates for viral TPK corresponding to this size have been described in other cellular systems ; p55 could thus represent a substrate for tyrosine phosphorylation specific to lymphoïd cells.

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