Protein kinase activation in *Theileria*-infected cells

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Theileria parva, a tick-transmitted protozoan parasite, is the causative agent of theilerosis, an often fatal lymphoproliferative disease of livestock in East and Central Africa. The related parasites *T*. *annulata* and *T*. *sergenti*, cause similar diseases in the Middle East/India and the Far East, respectively. Although *T*. *parva* sporozoites may enter a variety of bovine cell types, they appear to be able to survive only in subpopulations of lymphocytes in which the parasite differentiates into a multinucleate schizont.[1] *Theileria* schizonts are unique among protozoan parasites in that they induce lymphoblastogenesis and clonal expansion of quiescent target cells. Regardless of pre-infection phenotype, the *Theileria*-infected cells acquire some surface antigens characteristic of proliferating bovine T cells.[14] Since such cells are immortalized and can easily be propagated *in vitro*, exhibit cellular pleomorphism and appear to acquire a variety of other alterations in surface phenotype,[14] *Theileria*-infected cells have been considered transformed. However, such terminology has been considered misleading since it implied an oncogenic aetiology and since the infection is curable with administration of appropriate antibiotics.

Protein kinase activation in *Theileria*-infected cells

Ample evidence has been presented to implicate phosphorylation/dephosphorylation in the modulation of a large number of processes vital to the life of a cell. Among these are cellular differentiation, signal transduction and cellular proliferation.[2-9] Cells can proliferate in response to external signals as well as to intracellular events. Membrane receptors for several polypeptide hormones and growth factors have been shown to have endogenous tyrosine-specific protein kinase activity.[8-11] Upon receptor engagement by ligand, activation of the receptor kinase occurs, which initiates a series of modifications of specific target molecules, which are thought to constitute intracellular pathways for cellular growth (for review, see 8). Under normal circumstances, when a signal for cell growth ceases, homeostatic regulation ensures inactivation of important molecules or the reversion of their levels to those obtained at quiescence. It is thus envisaged that uncontrolled cellular growth could occur if important molecules regulating cellular proliferation are constitutively produced or if because of structural alterations, such molecules cease to be responsive to physiological control.

The important roles played by protein kinases in growth of cells stimulated us to determine if any differences existed between the activities of these enzymes in cloned bovine lymphocytes before and after infection with *Theileria* sporozoites. Supernatant and particulate fractions[3] (100,000 × g) prepared from IL-2 maintained, con A stimulated or normal peripheral blood

lymophocytes (PBL) were used as control for those from *Theileria*-infected cells. Most enzymatic activity was in the 100,000 × g particular fractions and was capable of phosphorylating both endogenous and exogenous substrates.

Analysis of target amino acids residues[13] and kinase co-factor requirements showed that the *Theileria*-associated kinase(s) were cyclic nucleotide independent, Ca^{++} calmodulin insensitive and phosphorylated serine/threonine, rather than tyrosine, residues on endogenous protein substrates. Among a large number of exogenous substrates examined, phosvitin, glycogen synthase and a wide variety of casein variants were the best substrates.

The susceptibility of the *Theileria*-associated kinases to inhibition by glycosaminoglycans, 2,3-bisphosphoglycerate, pyridoxal 5'-phosphate and to stimulation by polyamines suggests that the dominant protein kinase activity in *Theileria*-infected cells is casein kinase II-like.[6,7] Furthermore, employing anti-bovine casein kinase II antibody,[5] we have shown that the *Theileria*-infected cells have markedly increased amounts of casein kinase II antigen. Also the enzymatic activity is susceptible to partial inhibition by this antibody. Interestingly enough, in addition to a /a[1] and b subunits of the bovine casein kinase II, [5,17,18] we also see strongly reacting antigens in the Mr range of 15-20 kDa. The latter have only been seen so far in *Theileria*-infected cells. The latter findings are in agreement with our finding a casein kinase II-like enzymatic activity in purified *Theileria*-macroschizont preparations.[19] Current efforts are directed at structural and functional characterization of the parasite enzyme.

Conclusions

(1) Our findings of substantial increases of casein kinase II-like enzymatic activity, as well as antigen, in *Theileria*-infected cells suggest a possible role for this enzyme in *Theileria*-induced cellular proliferation. This suggestion is supported by recent findings showing:

(a) Brisk activation of casein kinase II in response to hormonal stimulation of cells *in vitro*.[17]

(b) Elevation of casein kinase II activity and antigen in differentiating cells.[18]

(c) Significant structural homologies between casein kinase II from various sources and the products of the cell cycle controlling genes in yeast and other species.[12,15,16]

(2) Induction of uncontrolled cellular proliferation by an intracellular parasite may thus obviate the need to invoke exogenous factors. The *Theileria* schizont may activate a pathway that the cell engages in response to normal growth signals. Uncontrolled growth may thus occur if *Theileria* drives the lymphocytes to constitutively produce normal growth signal-transducing molecules or, alternatively, if *Theileria*-derived analogs of molecular constitutents of intracellular pathways for growth may not be susceptible to modulation by host-cell elements.

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