ON THE KINETICS AND OPTIMAL SPECIFICITY OF CYTOTOXIC REACTIONS MEDIATED BY T-LYMPHOCYTE CLONES

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Using the chromium release assay and the single cell assay in agarose, we study the cytotoxic reaction of the MHC-restricted T lymphocyte clones P89:15 and P1:3, which recognize distinct but specific tumour antigens on the surface of syngeneic P815 mastocytoma cells. We propose a mathematical model which describes these experiments, accounts for the strongly non-Michaelian behaviour of the reaction and permits us to estimate the kinetic parameters characterizing effector-target conjugation and lethal hit delivery. The results show that the binding and lytic activity of effector cells is modulated by the number of targets bound to them. The binding of a second target by an effector having already a target bound is facilitated; on the other hand, an effector having bound two targets delivers a lethal hit more slowly than one with a single target bound. We investigate the role of these kinetic properties in the competition between the process of tumour progression due to cancer cell replication and the process of tumour regression due to T lymphocyte cytotoxic activity. For both clones, we estimate the effector-target ratio beyond which rejection prevails. This ratio is nine times larger for P1:3 than for P89:15. Furthermore, our analysis suggests that there exists an optimal specificity minimizing this ratio. Deviations from this optimum, be it in the sense of an increase or decrease of specificity, tends to stabilize the tumoural state: a situation which in the broader context of the immune response evolution and regulation can be viewed as an immune response dilemma.

1. Introduction. Cytotoxic T lymphocytes (CTL) are effector cells which form cellular conjugates with tumour cells and kill them by releasing a toxic agent or a messenger triggering a cascade of events ending by the target cells death (for a review on lymphocyte mediated cytolysis see Young and Liu, 1988). These processes of conjugation and lethal hit delivery are the main steps of a cytolytic cycle which may be sketched as: effector + target → effector-target-complex → effector + dead target.

This reaction scheme recalls that of Michaelian enzymes. Owing to this analogy, it has been supposed that lysis obeys the Michaelian rate law:
\[ v = \frac{v_{\text{max}} T}{(K_m + T)} \]

where the velocity, \( v \), represents the number of targets killed per unit time, \( T \) the number of targets initially present, \( v_{\text{max}} \) and \( K_m \) are the cellular analogs of the maximal enzymatic reaction rate and of the Michaelis constant. Expressions like this have been proposed for a wide variety of effector cells of murine and human origin, e.g. allogeneic CTL, activated macrophages, natural killer cells (NK), lymphokine-activated killer cells (LAK) (Thorn and Henney, 1976; Zeijlemaker et al., 1977; Callewaert et al., 1978, 1985; Garay and Lefever, 1978; Lefever and Garay, 1978; Pollack and Emmons, 1979; Merrill, 1982; Lefever et al., 1986, 1989).

Let us situate this Michaelian approach of cell mediated cytolysis (CMC) more precisely. The Michaelian law is an approximation valid for large times when the initial transients of an enzymatic reaction have relaxed. Its derivation, starting from enzymatic kinetic equations, is based on the so called steady state assumption. What underlies this notion is a separation of time scales: the substrate (slow variable) must evolve slowly compared to the enzymatic forms (fast variables). With cellular cytotoxic reactions, the formulation of kinetic equations furnishing a relevant starting point for deriving the Michaelian approximation is a problem in itself. Typically the Michaelian approach of CMC dodges this problem. It proceeds tentatively as follows: (i) it is expected that the steady state assumption, and hence the Michaelian law, hold provided that the rate of cytolysis be constant in time, which is often the case during several hours in in vitro experiments; (ii) the data obtained while this condition is met, are then tested to determine whether indeed they conform with the properties of a Michaelian kinetics; and (iii) if not, nevertheless, the Michaelian law is adopted but with some adaptation allowing the experimental results to fit, e.g. by letting \( K_m \) depend in a more or less complicated way upon the effector cells concentration. At the same time that it avoids the problem of dealing with more complicated kinetic equations, this procedure appeals because it involves only two parameters (\( v_{\text{max}} \) and \( K_m \)) which are easily evaluated by the standard technique of Lineweaver–Burk plots. This is convenient to describe the effects due to changes in experimental conditions or in cellular populations; furthermore \( v_{\text{max}} \) and \( K_m \) look familiar, thanks to the enzymatic analogy. These practical advantages explain the success enjoyed by this rather corner-cutting approach. They cannot, however, smooth off the shortcoming that, at the starting point, it strongly leans upon the steady state assumption: neither the condition that the lytic rate be constant, nor even the fact that Michaelian fits seem adequate, are evidence sufficient to legitimate the expectation that the steady state assumption and the Michaelian law really hold, or put another way, that a mathematically sound derivation of this law be actually possible. In fact, as we indicate in the next paragraph, an examination of the literature data shows the contrary. We conclude therefore that the Michaelian expressions proposed so far are empirical. As such, they cannot serve to estimate