sarcomas. Similarly, metastases, that is, tumour recurrences found at a site distant from the original tumour, may contain antigens different from those of the primary tumour, or may have lost some antigens completely — the so-called deletion mutants, for example (Sugarbaker & Cohen *Surgery* **72**, 155; 1972).

The significance of such immunological heterogeneity should not be lost on those who use established transplantable tumours to study the immunology of the tumour–host relationship. What it means in relation to the specific immunotherapy of experimental tumours is made abundantly clear by some recent experiments reported by Olsson and Ebbesen (*J. natn. Cancer Inst.* **62**, 623; 1979). They attempted to prevent the growth of AKR mouse thymic lymphomas (of recent origin), by repeatedly injecting young mice with irradiated AKR thymoma cells, after the recipients had been injected with untreated cells of a primary AKR thymoma. Not surprisingly, the procedure was generally unsuccessful; about 70% of the treated mice developed thymomas. What was surprising was that the thymomas which arose in these mice were immunologically distinct from those in untreated control recipients, as assessed by antibody and cytotoxic T cell assays. After analysing many tumours, the authors concluded that virtually all AKR thymomas are immunologically polyclonal, consisting of a dominant clone, and three distinct minor subclones — the latter group comprising less than 3% of the original tumour cell population. By treating animals with unfractionated thymoma cells, effective immunity is induced only against the dominant clone since the cells of any minor clone are present in too small a number to stimulate the immune system. This merely allows the cells of a minor or 'silent' clone to flourish, so that it eventually becomes dominant. When Olsson and Ebbesen treated their animals with a mixture of equal and large numbers of cells from each clone, so that effective immunity would be produced against all the clones, they produced a cure rate of greater than 90%.

These results also suggest that tumour progression may be more complex than previously thought. It may be that newly emerging clones do not always have to replace one another successively or die off. Rather, many may lead a chronic but cryptic existence, content to live in the shadow of the dominant clone. Only with a favourable change in the immunological *status quo* do they get a chance to flex their muscles.

It would be tempting to blame the failures of specific tumour immunotherapy on immunological heterogeneity and the existence of 'silent' subclones. There are, however, many who would say this is nonsense since most naturally arising tumours are not demonstrably immunogenic (Hewitt *et al. Br. J. Cancer* **33**, 241; 1976). Indeed, the Kleins have stressed the importance of the unrelenting process of tumour progression in the evolution of non-immunogenic spontaneous tumours, whereby successive immunogenic clonal variants replace each other as a result of selection, until a variant emerges which, lacking tumour antigens, becomes independent of the usual restrictions imposed by the immune system (*Proc. natn. Acad. Sci. U.S.A.* **74**, 2121; 1977). This remains a sensitive issue, and the antigenic properties of human tumours are still far from clear. Perhaps the extensive use of hybridomas to produce monoclonal antibodies against putative human tumour-specific antigens will help eventually to resolve this controversy (Herlyn *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 1438; 1979).

The non-immunogenicity of many primary spontaneous tumours and the polyclonal nature of those that are immunogenic, do not auger particularly

Is NGF an enzyme?

NERVE growth factor (NGF) is a protein with a key role in the growth and differentiation of immature sympathetic nerve cells and in the maintenance of fully differentiated sympathetic neurones.[1] Since its discovery in several organs, cell lines and body fluids, biochemists have faced the apparently simple but actually very difficult task of isolating and identifying the polypeptide responsible for such impressive biological action. Attempts to purify this 'molecule' from cell-free extracts have been hampered by the tendency of NGF to associate with other components of the homogenates. Thus the apparent size and sedimentation constant of the NGF 'molecule' have varied according to isolation procedures used. The molecular identity of NGF has changed from a protein of molecular weight 44,000 (ref. 2) to a component of molecular weight 40,000 which is strongly associated with other components of the extract so that together they elute as a protein larger than 100,000 (ref. 3). A form of NGF defined as 7S on the basis of its sedimentation behaviour has also been isolated and found to be[4] a mixture or complex of three proteins defined as α, ß, γ. While γ is an enzyme with arginine esteropeptidase activity and α has no detectable biological or biochemical action, β is the only component of the complex endowed with nerve growth promoting acitvity. Subsequent studies suggested that the function of the γ component in an intact cell is to cleave a pro-NGF chain of larger size, as demonstrated for hormones such as insulin[5].

Finally, a 30,000 molecular weight NGF consisting of two identical polypeptide chains held together by non-covalent bonds was isolated in pure form and defined as 2.5S NGF[6]. The β and 2.5S NGF are almost identical proteins differing only in an N-terminal octapeptide with no detectable relevance to nerve growth promoting activity. This latter preparation has been sequenced and studied extensively[7]. All attempts to detect enzymatic activity in 2.5S NGF have given negative results.

In a recent article NGF has been reported to be a specific protease although the experiments supporting this conclusion leave room for doubt[8]. The authors isolated from the same source as that of the 2.5S or β NGF, the mouse salivary gland, a protein(s) with molecular weight of 116,000. In acrylamide gel electrophoresis in the absence of detergents or other denaturing substances, this protein(s) runs as a single band which exhibits a protease activity with an almost evanescent degree of specificity for plasminogen (880 ng of this preparation have an activity comparable with 0.083 ng of urokinase). The same band cross-reacts with antibodies directed against 2.5S NGF, a finding which is taken as evidence that NGF is a protease. The authors have overlooked the possibility that the apparently homogeneous 116,000 molecular weight component with protease activity, may be a complex of 2.5S or β NGF plus some other protein(s) easily identifiable with the many proteases present in the salivary glands. Their conclusion requires support from evidence obtained in carefully controlled dissociating conditions that the 116,000 molecular weight complex is indeed composed of a single polypeptide chain and is not a mixture of at least two different entities: one with properties of the 2.5S or β NGF but devoid of enzymatic activity and the other(s) with protease activity. The same authors while reporting this enzymatic property in the 116,000 molecular weight protein acknowledge that the 2.5S NGF alone does not possess such activity. Although all previous work indicates that NGF is associated in mouse salivary glands with proteases, this association is not evidence that NGF itself is an enzyme.

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1. Levi-Montalcini R. *Harvey Lectures*, **60**, 217 (1966).
2. Cohen S. *Proc. natn. Acad. Sci. U.S.A.*, **46**, 302 (1960).
3. Angeletti P.U., Calissano P., Chen J.S. & Levi-Montalcini R. *Biochim. biophys. Acta*, **147**, 180 (1967).
4. Varon S., Nomura J., Shooter E.M. *Proc. natn. Acad. Sci. U.S.A.*, **57**, 1782 (1967).
5. Berger E.A. & Shooter E.M. *Proc. natn. Acad. Sci. U.S.A.*, **74**, 3647 (1977).
6. Bocchini V. & Angeletti P.U. *Proc. natn. Acad. Sci. U.S.A.*, **64**, 787 (1969).
7. Hogue Angeletti R. & Bradshaw R.A. *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2417 (1971).
8. Orenstein N.S., Dvorak H.F., Blanchard M.H. & Young M. *Proc. natn. Acad. Sci. U.S.A.*, **75**, 5497 (1978).

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