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Special Article

Lymphotoxins, Macrophage Cytotoxins, and Tumor Necrosis Factors: An Interrelated Family of Antitumor Effector Molecules

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INTRODUCTION

It has been known for a number of years that activated lymphocytes and macrophages from experimental animals and humans can be stimulated *in vitro* to release materials that are growth inhibitory and lytic for nucleated mammalian cells *in vitro*. Materials with these activities were termed lymphotoxins (LT) and macrophage cytotoxins (MCT), respectively (1, 2). It is also evident that serum from bacillus Calmette Guerin (BCG)-infected and endotoxin-challenged animals contains a material(s) that causes selective necrosis of murine tumors when injected into tumor-bearing mice. The active component(s) in these sera was termed tumor necrosis factor (TNF) (3, 4). Recent results show that MCT and LT are discrete but related proteins. Both of these materials selectively affect transformed cells *in vitro* and both have TNF activity in the murine tumor model. These results unite *in vitro* and *in vivo* phenoma and suggest that both lymphocytes and macrophage may share a common mechanism(s) for the control and destruction of transformed cells. Finally, there is the possibility that these effectors will provide a new means for anticancer therapy.

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LYMPHOTOXINS

It was reported in the late 1960s and early 1970s that lymphocytes from experimental animals and humans could be stimulated to release lymphotoxins *in vitro* (1). It quickly became apparent that the effects of LT on cells *in vitro* were not species specific and that different cell lines each had a unique susceptibility to the materials. Sensitive cells were lysed in 16–48 hr and resistant cells were unaffected or either reversibly or irreversibly growth inhibited. Because of their sensitivity, murine L-929 cells were routinely employed as indicator cells in the *in vitro* assays employed by most investigators. Biochemical studies revealed that lymphotoxins are heterogeneous proteins that are separable into different molecular weight (MW) classes ranging from 15,000 to 1.5×10^6. One set of different MW forms from lectin-stimulated human lymphocytes was shown to be composed of interrelated subunits. The 70,000–90,000 MW form from this interrelated family was termed alpha LT. Subsequent studies revealed that the alpha LT could be released by both T- and B-lymphocyte subpopulations and by continuous lymphoblastoid cell lines. However, it was subsequently shown that NK and CTL effector cells can release different LT forms. Human alpha LT from lymphoblastoid cell lines has been purified to homogeneity and exists as two different molecular forms. In supernatants from RPMI-1788 cells it is a 70,000 MW molecule which is an assemblage of a single 20,000 MW peptide (5).

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However, in supernatants from GM3104A cells it is a 90,000 MW form composed of a 20,000 MW peptide associated with a 68,000 MW peptide (6). The small peptide appears to be identical in the alpha molecule from both cell lines.

In the mid-1970s it was recognized that LT-containing preparations have selective effects on transformed cells *in vitro* and also affect the growth of tumors *in vivo*. While these studies were provocative, they uniformly suffered from the use of unpurified materials, and thus the observed antitumor effects could not be attributed solely to LT. However, certain of the results from these early studies appear to be correct, for they have recently been verified with purified human LT forms.

MACROPHAGE CYTOTOXIC FACTORS

Macrophages and certain continuous macrophage-like cell lines, from experimental animals and humans, can be stimulated to release materials with MCT activity *in vitro* (2, 3). However, these effector cells and cell lines must first be raised to a tumoricidal state of activation before they can be stimulated to release MCT. Similar to LT, MCT effects on cells *in vitro* are not species specific, do not affect all types of cells, and require from 16 to 48 hr to become evident. Recent results from a number of laboratories has shown that human and murine MCTs are more effective on transformed than primary cells *in vitro*. Biochemical studies of MCT revealed that they are proteins that vary in size from 50,000 to 160,000 MW. Several studies indicate that MCT activity from human macrophage cell lines is blocked by serine protease inhibitors *in vitro*. Immunologic studies indicated that human MCT is not related to human alpha LT. In contrast, MCT and LT from mice and guinea pigs were shown to be immunologically cross-reactive.

TUMOR NECROSIS FACTORS

While the phenomenon of tumor necrosis was recognized earlier, the observation that serum contained the TNF activity was first reported in the mid-1970s (3, 4). Serum from BCG-infected, endotoxin-stimulated animals was shown to cause hemorrhagic necrosis of murine tumors when administered to tumor-bearing animals. The material was effective when injected directly into the tumor or when administered intravenously into the animals. Necrosis was evident after 16–24 hr. It was subsequently shown that serum containing TNF caused cell lysis *in vitro,* and because of their sensitivity L-929 cells were routinely employed as *in vitro* target cells to detect these materials. Biochemical studies revealed that TNF are proteins from 50,000 to 160,000 MW. It was demonstrated by immunologic methods that the material in serum active in the TNF assay and the material that lysed L929 cells *in vitro* were related. Employing similar techniques, it was demonstrated that TNF and MCT from activated macrophages were also related. These and other findings lead to the premise that macrophages were the *in vivo* cell source of the serum TNF activity. However, recent studies described a material(s) with TNF activity *in vivo* that was present in the supernatant from various human lymphoblastoid cell lines (7). These supernatants also had selective effects on certain transformed cells *in vitro*. The TNF from the human lymphoid cells lines appeared to be biochemically and functionally similar to alpha LT. This was not surprising for earlier studies, employing the same *in vitro* L-cell assay, had shown that alpha LT was the only toxin in these same supernatants. Thus, the concept was emerging that materials being studied as LT and MCT could be releated to the materials termed TNF.

CLONING OF HUMAN MCT AND LT AND RELATIONSHIP TO TNF

Recent data, obtained by recombinant DNA technology, show that human MCT and alpha LT are distinct but related molecules and that these proteins both have TNF activity *in vivo*. Scientists at Genentech purified, sequenced, and cloned the alpha LT molecule from RPMI-1788 cells and MCT from the human macrophage line HL-60 (7, 8). They found that MCT was formed by the assembly of a 20,000 MW peptide and that the amino acid sequence of the 20,000 MW LT peptide shares a 28% homology with the MCT peptide. They then demonstrated that both recombinant and native LT and MCT forms are active in the murine TNF assay system. Shortly after this report scientists at the City of Hope reported the cloning and sequencing of MCT from a different continuous human macrophage cell line (9). DNA and protein sequencing revealed that the material was essentially identical to the MCT reported by Genentech. This

material is also active in the TNF assay in the murine system. An important aspect of this work was that the DNA probe used to isolate the human MCT molecule was actually produced from an amino acid sequence of TNF obtained by the traditional method from rabbit serum. Thus, the human MCT molecule was directly related to rabbit TNF.

Our own data indicate that there are other human LT forms that can have TNF activity. The alpha LT form from GM3104A cells, which is composed of heavy and light chains, is active in the murine TNF assay. We have also purified a second human LT form released by a continuous human T-cell line which is a single peptide of 68,000 MW. This protein expresses antigenic determinants of both MCT and alpha LT and is much more active on transformed cells *in vitro* and in the murine TNF assay system than either MCT or LT alone.

CONCLUSION

Collectively these data present several new and exciting concepts. It is apparent that stimulated lymphocytes and macrophages can release similar but distinct proteins, LT and MCT, respectively. Recent data indicate that certain lymphocytes may also be able to release MCT-like molecules. These proteins can all cause necrosis of tumors in the murine system. These results support the concept that these are the cells responsible for the release of *in vivo* TNF activity. In studies of these forms it becomes very clear that they are more effective *in vivo* than *in vitro* by many orders of magnitude. These results suggest that they may function in collaboration with other host mechanisms to cause the TNF tissue destructive reaction(s). *In vitro* studies have shown that these effector molecules are released when NK, CTL, and macrophages interact with transformed cells. Thus it is logical to surmise that a small number of host effector cells could induce a significant amount of tissue destruction by the release of small amounts of these substances in an actual tumor site. Now that adequate quantities of purified factors and antibodies to these materials are available, how they exert selective effects on transformed cells *in vitro* and *in vivo* can be examined. How these molecules selectively affect tumor cells may provide new and fundamental information about the differences between transformed and normal cells. It should not be forgotten that these effectors may also have other important functions, for even the present data indicate that human alpha LT can (a) synergize with alpha, beta, and gamma human IFN *in vitro* to cause increased lysis of transformed cells; (b) activate macrophages and PMN cells; and (c) destroy virus-infected cells. Finally, they hold the promise, largely unfulfilled by interferon, for a new type of anticancer therapy. Needless to say, the next few years should yield some very interesting results about these effector molecules.

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