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Tumor Necrosis Factor: A Potent Mediator of Macrophage-Dependent Tumor-Cell Killing *

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A. Introduction

Macrophages (Mϕ) can be activated to show highly selective cytotoxicity toward malignant cells in vitro [6, 8, 9, 13, 14] and there is some evidence that they may destroy neoplastic cells in vivo [1]. The importance of activated Mϕ (aMϕ) in controlling tumor growth in vivo has been further implicated in experiments involving murine ultraviolet light (UV)-induced tumors, which are highly immunogenic regressor tumors [10] sensitive to Mϕ in vitro [22]. Variants of these tumors demonstrating progressive growth in the normal host were found to invariably express an increased resistance to aMϕ [22]. Furthermore, exposure of regressor tumor cells to aMϕ in vitro also resulted in selection for Mϕ-resistant cancer cells which displayed an increased early growth potential in vivo [22]. More recently we have utilized these tumor variants resistant to aMϕ to explore the mechanism by which aMϕ induce tumor cell destruction [23]. Our results suggest a major role for tumor necrosis factor type α (TNF-α) in Mϕ-mediated tumor cell killing in vitro and in vivo [23].

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B. Methods

Mϕ were peritoneal exudate cells obtained from thioglycollate-primed C3H/HeN (MTV$^-$) mice, activated in vitro for 6 h with lipopolysaccharide and lymphokine and used as effectors in a 16-h ^{51}Cr release assay, a 72-h ^{51}Cr postlabelling assay, or a 72-h [^{3}H]-thymidine release assay as described [22, 23]. C3H/HeN (MTV$^-$) mice were obtained from the National Cancer Institute, Frederick Cancer Research Facility. The UV-induced tumors 1591-RE and 2240-RE were induced in these mice by M. L. Kripke [10]. Human recombinant (r) TNF-α [18], B-cell lymphotoxin (TNF-β) [7], murine rTNF-α [19], polyclonal rabbit antibody to murine rTNF-α, and monoclonal antibody to human rTNF-α were produced at Genentech (South San Francisco, CA). Recombinant murine interleukin 1 (IL-1) [12] was kindly provided by Hoffman-LaRoche.

C. Results

Mϕ are known to secrete a number of different cytotoxic substances, including interleukin 1 (IL-1) [16], reactive oxygen intermediates, such as hydrogen peroxide [15] and TNF-α [5, 18, 21]. To test each of these as potential mediators of Mϕ-dependent tumor cytotoxicity, we analyzed each for preferential killing of the 1591 parent tumor over several of its Mϕ-resistant variants. Figure 1 shows that of these substances, only human rTNF-α demonstrated selective killing of the parent tumor over Mϕ-resistant variants isolated in vitro (panel d) or in

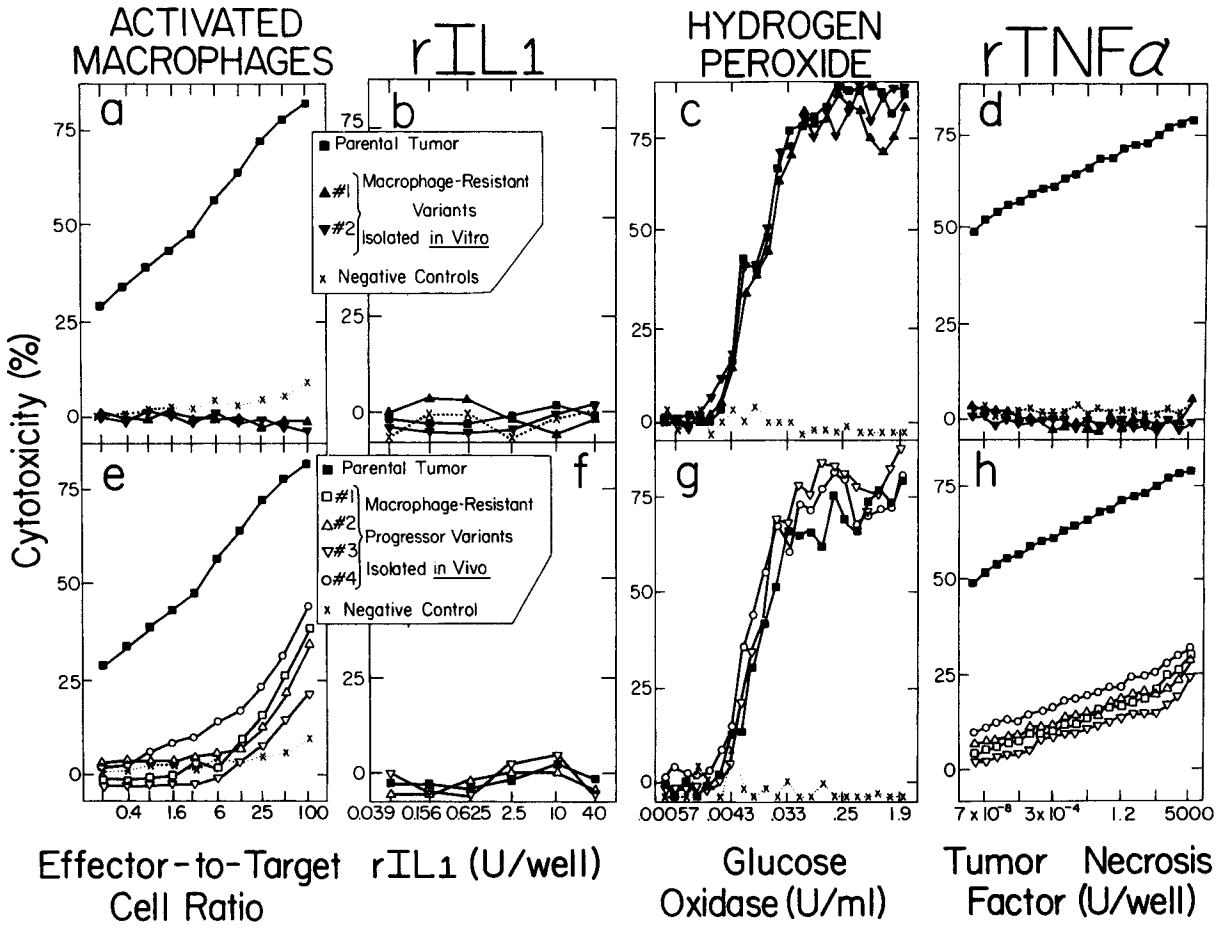


Fig. 1 a–h. Sensitivity of Mϕ-resistant 1591 tumor variants to soluble mediators of cytotoxicity. Results utilizing Mϕ-resistant variants selected in vitro are shown in **a–d** and results with variants selected in vivo are shown in **e–h.** Mϕ were activated as described [23] and used as effectors in a 16 h ^{51}Cr release assay (**a** and **e**); 10T1/2 fibroblasts were used as negative controls. Murine rIL-1 was quantified using a thymocyte proliferation assay [12] with heat-inactivated IL-1 used as a negative control. Hydrogen peroxide was generated using glucose oxidase [15] with 1 unit defined as the generation of 1 μmol H_2O_2 per min. Catalase added at 40 units/well served as the negative control. Susceptibility to human rTNF-α was analyzed in a 72 h ^{51}Cr postlabelling assay [22]. The negative control consisted of preincubation with monoclonal anti-TNF-α antibody at 1.85 μg/ml for 16 h. The data represent pooled values from three separate experiments with the SEM for each point indicated as ≦10% of the value of each point shown [23]

vivo (panel h). This closely mimicked the action of aMϕ themselves on these targets (Fig. 1, panels a, e). Furthermore, the effects of human rTNF-α on 1591 were completely neutralized by preincubation with a monoclonal antibody directed against human rTNF-α (Fig. 2 d, negative control). The resistance of the variants to aMϕ and human rTNF-α was selective in that the variants were fully sensitive to the effects of osmotic lysis, natural killer cells, and cytolytic T cells [23].

To confirm the linkage between resistance to human rTNF-α and resistance to aMϕ, two human rTNF-α-resistant 1591 cell lines were selected and tested for resistance to aMϕ. Figure 2 a shows that these human rTNF-α-resistant variants were substantially more resistant to aMϕ than was the parental 1591 tumor. The small residual sensitivity of the variants to aMϕ was completely abrogated by selecting with murine rather than with human rTNF-α (Fig. 2 a). Additional evidence to suggest that the observed cytotoxic effects of aMϕ and TNF-α follow identical pathways is given in Fig. 2 b. Increasing concentrations of a polyclonal antibody that neutralizes murine rTNF-α inhibited aMϕ killing of 1591 in a dose-dependent fashion, whereas incubation of aMϕ with preim-

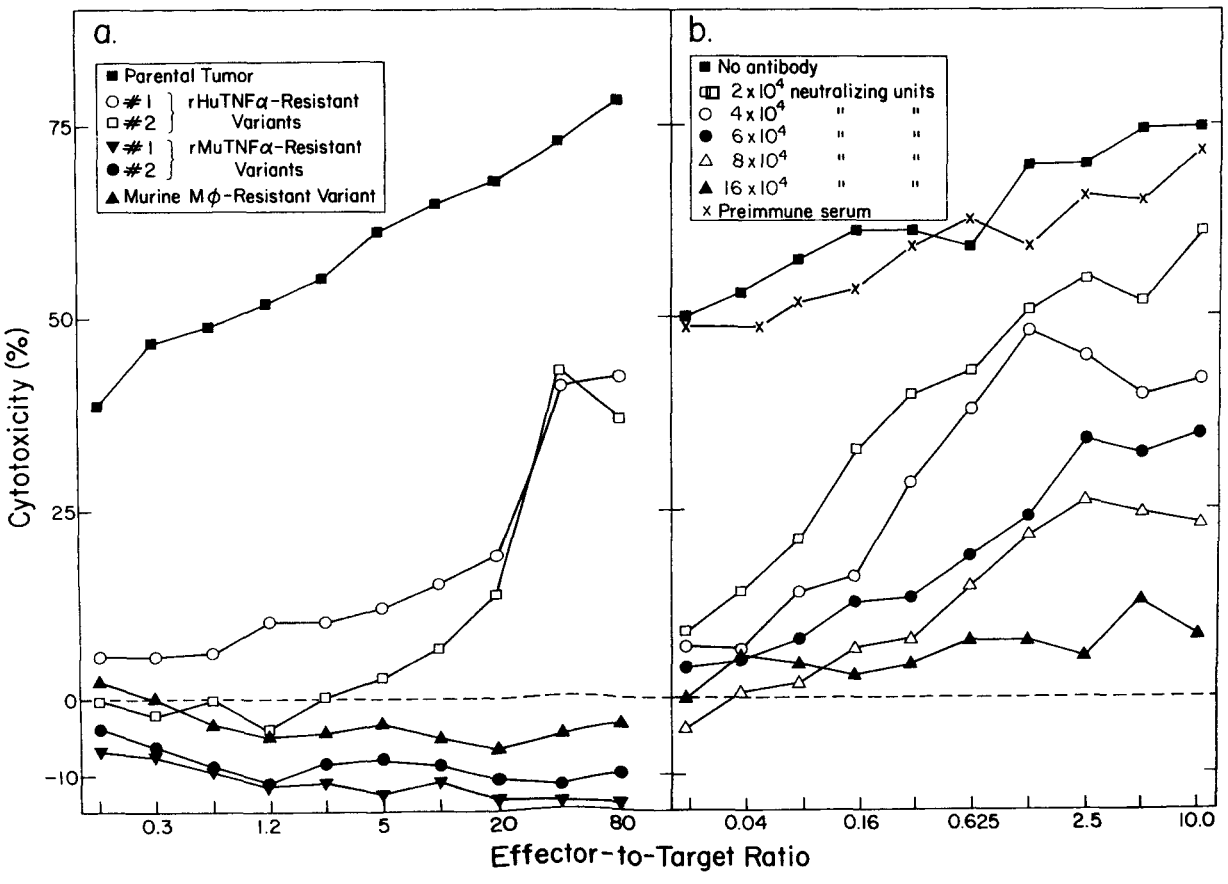
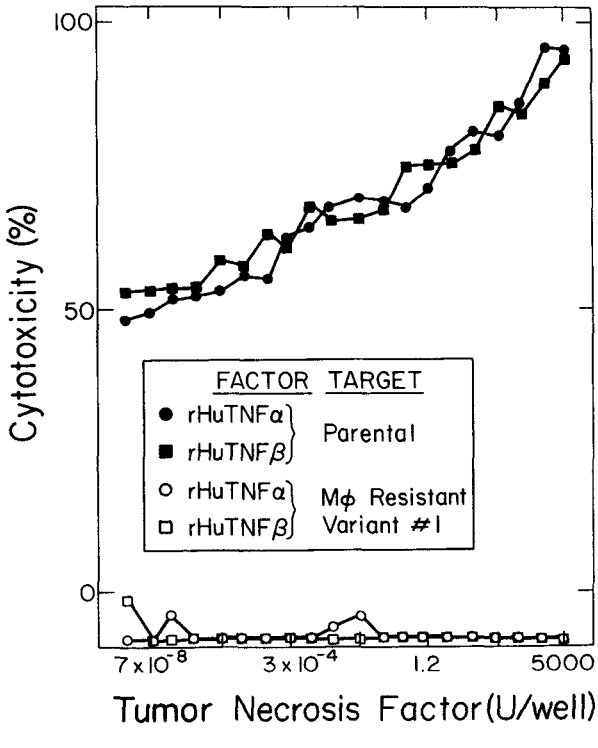


Fig. 2. **a** Complete resistance of the variants selected with murine rTNF-α to the cytolytic effects of aMϕ. Variants selected with human rTNF-α show only partial resistance. **b** Neutralization of Mϕ-mediated tumor cytotoxicity using rabbit polyclonal antibody against murine rTNF-α. Data represent the pooled results from two experiments using a modified ^{51}Cr release assay [22, 23]



mune serum resulted in a cytotoxic response similar to that of aMϕ alone.

Human TNF-β is a cytotoxic protein whose sequence is about 30% homologous to human TNF-α [2]. Figure 3 shows that human TNF-β was identical to human TNF-α in exerting a potent selective cytotoxic effect on the parental 1591 tumor over the 1591 Mϕ-resistant variant. This result raises the possibility that TNF-α and TNF-β employ common effector pathways, a suggestion consistent with other data indicating

Fig. 3. Resistance of the Mϕ-selected 1591 tumor variant to the cytotoxic effects of human rTNF-α and human rTNF-β. The parental 1591 tumor cells are equally sensitive to both recombinant proteins in a 72 h ^{51}Cr postlabelling assay. The data represent pooled values from two separate experiments [23]

that human rTNF-α and human rTNF-β compete for the same cellular receptor [3].

D. Discussion

Our results strongly suggest that TNF-α is an important effector molecule mediating Mϕ-dependent tumor cytotoxicity. All of the classical tumoricidal effects of aMϕ we observed on the 1591 tumor could be accounted for by TNF-α released from aMϕ. This was substantiated by the evidence that antibody to murine rTNF-α blocked the tumoricidal effects of aMϕ. Furthermore, selection with either aMϕ or murine rTNF-α led to simultaneous resistance to both aMϕ and TNF-α, but not to resistance to other tumoricidal mediators including IL-1 and hydrogen peroxide. The fact that these variants also retained their sensitivity to NK cells and cytolytic T cells [23] is consistent with other data suggesting that these cytolytic effector cells act through a lytic mechanism distinct from that of aMϕ [1].

Mϕ-resistant tumor variants isolated in vitro have been shown to display enhanced growth in the normal host [22], but the role of aMϕ in destroying or inhibiting nascent tumor cell growth is not fully understood. Furthermore, the precise mechanism by which TNF-α from aMϕ reaches the target cell remains unknown. In vivo, cell-to-cell contact may be required to prevent rapid diffusion and to assure a sufficiently high local concentration of TNF-α in the narrow space between the aMϕ and the bound target cell, while in vitro contact may only be required for less sensitive target cells.

The variants we have derived from selection with either aMϕ or rTNF-α retain their phenotype through prolonged passage in vivo or in vitro and it is clear that the resistance is heritable and may, therefore, have a genetic basis. Whether resistance to TNF-α may be associated with a decrease in the number of TNF receptors on the tumor cells has been investigated [4, 11, 20]. The variants we have described provide a new tool with which to dissect the precise mechanism of Mϕ-mediated cytotoxicity and to uncover the molecular and genetic mechanisms of malignant transformation leading to susceptibility to aMϕ. A study of these variants should also provide insight into how tumor cells become resistant to aMϕ and TNF-α and how we might overcome this resistance.

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