Studies of Connective Tissue Mast Cell-Mediated Cytotoxicity

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Although mast cells have been implicated in mediating antitumor activity, the kinetics, mechanism(s), and suspectibility of different tumors to mast cell-mediated cytotoxicity have not been defined. Rat connective tissue mast cells (CTMC) of ≥99% purity were investigated in vitro and found to express maximal spontaneous cytotoxicity against the mouse fibrosarcoma cell line WEHI-164 (56.0% ± 2.1 SEM), the ultraviolet B (UVB)-induced, cutaneous fibrosarcoma 5C25 (34.7% ± 3.4 SEM), and the human renal cell tumor Currie (26.8% ± 2.0 SEM) at an effector to target (E:T) ratio of 80:1. Kinetic studies of CTMC-mediated cytotoxicity demonstrated significant detectable lysis against these tumors within 8 h, which was maximal by 16 h. Binding experiments showed that CTMC formed conjugates with all three lytic-sensitive targets; however, CTMC also attached to the lytic-resistant target YAC-1, indicating that conjugate formation alone is not sufficient for mast cell-mediated cytotoxicity. At two different concentrations, mast cell granules (MCG) lysed WEHI-164 (36.5% ± 6.8 SEM) and 5C25 (34.4% ± 6.9 SEM), but were only slightly cytotoxic (5.7% ± 2.9 SEM) against Currie. A potential role for tumor necrosis factor-alpha (TNF-α) in CTMC-mediated cytotoxicity also was investigated. Polyclonal antibodies to TNF-α greatly reduced CTMC and TNF-mediated lysis of WEHI-164, but only partially inhibited CTMC killing of the slightly TNF-sensitive 5C25 tumors, and had no effect on CTMC cytolysis of Currie. Thus, this study demonstrates that CTMC mediate cytotoxicity in vitro by both TNF-associated and TNF-independent mechanisms. We conclude that CTMC are capable of mediating antitumor activity and that this effect may be important for tumor surveillance in the skin and other sites. *J Invest Dermatol 93:423–428, 1989*

The tissue mast cell is recognized for its role in several different immune-mediated events including immediate hypersensitivity reactions as well as complement and lymphocyte associated inflammation [1–5]. Recently mast cells also have been implicated in mediating antitumor activity in vivo and in vitro. Studies performed in mast cell deficient (W/W^v) and normal congenic (+/+) mice have shown an inverse correlation between cutaneous mast cell content and the incidence of experimentally induced tumors [6,7]. In addition, stimulation of murine skin mast cells in fibrosarcoma-bearing animals has resulted in local tumor regression [8]. An antitumor role for human mast cells also has been suggested by reports demonstrating increased mast cells in histologic sections of neoplasms invading the skin and other organs [9–12].

Recent studies performed in rodents and humans have demonstrated the presence of two phenotypically and functionally distinct mast cell populations. These include connective tissue mast cells (CTMC), which have been identified in the skin, serosal cavities and muscularis propria of the stomach, and mucosal mast cells (MMC), which are present in the bone marrow, lung, and mucosal layer of the gastrointestinal tract [5,13–18]. Interleukin-3 (IL-3)-dependent, bone marrow-derived cells with morphologic characteristics similar to MMC have been reported to mediate antitumor activity in vitro within 18–20 h [19,20]. Connective tissue mast cells also have been shown to kill specific lymphoma cell and fibrosarcoma cell lines in vitro at 1 and 48 h, respectively [21,22]. Although the mast cell appears to be a potentially important effector of antitumor activity, the kinetics, mechanism(s), and tumor target

Manuscript received July 19, 1988; accepted for publication May 11, 1989.

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This work was supported by National Institutes of Health Grant-CA 48539.

Abbreviations:
BCC: basal cell carcinoma
BSA: bovine serum albumin
CTMC: connective tissue mast cells
E:T: effector to target ratio
FCS: fetal calf serum
HBSS: Hanks' balanced salt solution
Il-3: interleukin-3
KGM: keratinocyte growth medium
MCC: mast cell cytoplasts
MCG: mast cell granules
NC: natural cytotoxic
NK: natural killer
NU: neutralizing unit
SCC: squamous cell carcinoma
anti-TNF: antihuman tumor necrosis factor
TNF-α: tumor necrosis factor-alpha
r-TNF-α: recombinant tumor necrosis factor-alpha
UVB: ultraviolet B

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specificity of CTMC-mediated cytolysis have not been well-defined. Therefore, the purpose of this study was to investigate the characteristics of CTMC-mediated cytotoxicity in vitro.

MATERIALS AND METHODS

Reagents The reagents used and their sources were as follows: histamine dihydrochloride, porcine intestinal heparin, bovine serum albumin (BSA), HEPES, procine kidney diamine oxidase (histaminase), and gentamicin (Sigma, St. Louis, MO); RPMI 1640 medium and 10X Hanks' balanced salt solution (HBSS) (GIBCO Laboratories, Grand Island, NY); keratinocyte growth medium (KGM) (Clonetics Corp, San Diego, CA); fetal calf serum (Hazelton, Denver, PA); Percoll (Pharmacia, Uppsala, Sweden); ^{3}H-S-adenosylmethionine (New England Nuclear, Boston, MA); $Na_2{}^{51}CrO_4$ (ICN, Plainview, NY); Ready Solv scintillation cocktail (Beckman Instruments, Irvine, CA). Purified human recombinant TNF-alpha (r-TNF-α, Lot TD4) and rabbit polyclonal anti-human TNF antibodies (anti-TNF antibodies, Lot 57-030685) were generous gifts of the Cetus Corp. (Emeryville, CA). One unit (U) of r-TNF-α activity was defined as the reciprocal of the dilution at which 50% cytotoxicity against L929 tumor cells occurred after 18 h. The activity of anti-TNF antibodies was expressed in neutralizing units (NU), which were defined as the dilution of anti-TNF antibody-containing serum necessary to inhibit 1 U of TNF.

Cultured Cells The tumor cell lines, WEHI-164, P815, and the human erythroleukemia line, K562, were gifts from Dr. M. Bennett, University of Texas Health Science Center at Dallas (UTHSCD); the renal cell carcinoma line, Currie [23] was obtained from the laboratory of Dr. M. Prager, UTHSCD. The ultraviolet light induced cutaneous fibrosarcoma, 5C25 [24] was a gift from Dr. Jerry Niederkorn, UTHSCD. YAC-1 and HEp-2 were purchased from American Type Culture Collection (Rockville, MD). Human keratinocytes were purchased from Clonetics Corp (San Diego, CA). Cultures of human skin fibroblasts were obtained from freshly discarded neonatal foreskins using a combined enzymatic digestion technique [5] and maintained in RPMI-1640 medium with 10% fetal calf serum (FCS). Second passage cultures of keratinocytes and fibroblasts were used for cytotoxicity experiments.

Purified Rat Mast Cell Preparations Connective tissue mast cells were harvested from the thoracic and peritoneal cavities of Sprague-Dawley rats (Camm, Wayne, NJ) in MCM medium containing 150 mM sodium chloride, 3.7 mM KCl, 3 mM Na_2HPO_4, 0.9 mM $CaCl_2$, 5.6 mM dextrose, heparin (10 U/ml), and 0.1% BSA as described [25,26]. The resulting mixed cell suspension containing 5% – 8% mast cells was purified ≥ 99% homogeneity using a modified Percoll discontinuous gradient, flotation method of separation described by Enerback and Svensson [26]. Briefly, after spinning at 50 × g for 7 min and removing the buffer, the mixed cell population was resuspended in 1.0 ml of 9 parts Percoll and 1 part ×10 HBSS (90% Percoll), and transferred to a 15-ml conical tube. Two additional 1-ml gradients (80% and 70%) were sequentially layered over the cell suspension. After spinning at 400 × g for 12 min at 15°C, the upper two gradients were removed and discarded. Mast cells ranging in purity from 90% to ≥ 99% were recovered from the 90% gradient and washed twice in 15 ml of medium. Mast cell preparations of a ≤ 99% purity were enriched further to ≥ 99% homogeneity by repeating (×1) the separation procedure. At least 1000 cells were counted for the determination of mast cell purity. From 1.5 to 2.7 × 10^6 purified mast cells with a viability of > 95%, as determined by trypan blue exclusion, were routinely recovered from each animal. Before the experimental studies, purified mast cell preparations were maintained in RPMI-1640 medium with 5% FCS at room temperature.

Purification of Rat Mast Cell Cytoplasmic Granules Mast cell cytoplasmic granules (MCG) were prepared using a modified method of Raphael et al [27]. After purification, mast cells were suspended in 2 ml of buffer, sonicated (Kontes Glass Company Micro-Ultrasonic Cell Disrupter, Vineland, NJ) with a 4.5-inch probe at 9.92 W for 20 s, and gently centrifuged at 150 × g for 5 min. The granule-containing supernatant was removed and held at room temperature. The mast cell-containing pellet was resuspended in buffer and resonicated until the total number of intact mast cells was ≤ 1% of the starting population. Typically a total of 30 – 40 s of sonication was sufficient to lyse > 99% of the mast cells. The granule-containing supernatants were pooled and incubated in the presence of 2.5 mM EDTA for 15 min at 37°C. Granules were layered over a 2-ml sucrose solution (0.34 M), and centrifuged in a 15-ml conical tube at 450 × g for 10 min at 15°C. Purified MCG were recovered from the supernatant by centrifuging at 7800 × g for 20 min and washed twice in 1 ml of buffer. The granules were resuspended in RPMI medium with 5% FCS. As measured by histamine content [28], 50% to 75% of MCG were routinely recovered from the starting preparation using this technique.

Preparation of Mast Cell Cytoplasts Mast cell cytoplasts (MCC) were prepared as described [29]. Ten percent, 13%, and 25% Ficoll solutions containing 20 μM cytochalasin B were prepared from a 40% stock using phosphate-buffered saline (PBS) (140 mM NaCl, 10 mM sodium phosphate, pH 7.2). One milliliter of 25% Ficoll was added to the bottom of a 0.5 × 2-inch nitrocellulose ultracentrifuge tube (Beckman Instruments, Fullerton, CA) and 1 ml of a 13% Ficoll solution was subsequently added. Immediately after purification, mast cells were resuspended in 10% Ficoll, incubated for 5 min at 37°C, and carefully applied over the 13% and 25% Ficoll layers. The nitrocellulose tube was placed in a prewarmed (37°C) swinging bucket ultracentrifuge rotor (Sorvall AH-650) and centrifuged at 81,000 × g for 30 min at 33°C. Mast cell cytoplasts were recovered from the upper 1.25-ml fraction, sedimented in a microfuge (Beckman Microfuge-B), and counted. Previous work has demonstrated that cytoplast preparations of mast cells are devoid of nuclei and cytoplasmic organelles and that two MCC are generated from each mast cell [29].

Cytotoxicty Assay Mast cell-mediated lysis of different tumor targets was assessed by a standard ^{51}Cr release cytotoxicity assay. Tumor target cells were labeled with 200 μCi of $Na_2{}^{51}CrO_2$ in RPMI-1640 medium with 10% FCS for 90 min at 37°C in a 5% CO_2 environment. After washing, 5 × 10^3 labeled targets in 25 μl were added to microtiter plate U-shaped wells (Costar, Cambridge, MA) containing varying numbers of mast cells in a final volume of 200 μl to achieve effector to target (E:T) ratios ranging from 10:1 to 80:1. Each experimental and control condition was performed in triplicate. When the effects of immunologic agents on mast cell cytotoxicity were examined, the reagents also were added to targets alone as controls. At the end of the incubation period, a 100-μl aliquot of supernatant from each well was removed, and radioactivity was measured in an auto-gamma scintillation spectrometer (Packard Instrument Co., Downer's Gove, IL). The percent specific lysis was calculated by the following formula:

$$\% \text{ specific lysis} = \frac{\text{experimental release (cpm)} - \text{spontaneous release (cpm)}}{\text{total release (cpm)} - \text{spontaneous release (cpm)}} \times 100.$$

Total release refers to cpm obtained in wells containing 50% Titron X 100 as a cell lysing agent, and spontaneous release refers to cpm released by the targets incubated in the absence of mast cells. Spontaneous release for all tumor targets was routinely less than 30% at 16 h.

Mast Cell-Tumor Target Conjugate Formation Mast cell binding to different tumor targets was assessed over time (1 – 16 h). Quadruplicate cocultures of 5 × 10^4 mast cells and an equal number of tumor targets were suspended in 100 μl of RPMI-1640 medium and incubated for different time intervals in 0.5-ml microcentrifuge tubes (Westcoast Scientific, Emeryville, CA) at 37°C. At 1, 4, 8, and 16 h, 20-μl samples were gently removed from the mast cell-tumor target cocultures, stained with crystal violet and placed in a hemo-

cytometer. Mast cell-tumor target conjugates were enumerated by microscopic observation, and the percent mast cell binding was calculated by the formula:

$$\% \text{ mast cell binding} = \frac{\text{no. of mast cell bound to targets}}{\text{Total no. of mast cells counted}} \times 100.$$

At least 100 mast cells were counted in each quadruplicate sample at the designated times.

RESULTS

Tumor Target Specificity and Kinetics of Mast Cell-Mediated Cytotoxicity The susceptibility of both murine and human tumor cell lines to mast cell cytotoxicity was investigated by coculturing different targets with purified preparations of rat mast cells at three different E:T ratios (10:1, 40:1, and 80:1). Significant mast cell tumoricidal activity was detachable after 16 h against the murine fibrosarcoma cell line, WEHI-164, the UVB-induced murine, cutaneous fibrosarcoma tumor 5C25, and the human renal cell carcinoma Currie (Table I). Maximal mast cell cytotoxicity occurred at an E:T of 80:1 and ranged from 26.8% (±2.0 SEM) to 56.0% (±2.1 SEM). Only low levels of mast cell-mediated killing were observed against the human tumor cell lines, K562 and HEp-2, whereas no significant cytolysis was evident against the mouse tumors, YAC-1 and P815 (Table I). Similar experiments also showed that at an E:T of 80:1 mast cells did not kill cultured normal human skin fibroblasts (−1.9% ± 4.3 SEM) and induced minimal cytotoxicity against normal human keratinocytes (4.6% ± 0.7 SEM) in culture.

The kinetics of mast cell tumor killing also was examined, and after 4 h of coculture, only low levels of cytotoxicity were detectable against WEHI-164, 5C25, and Currie (E:T of 80:1) (Fig 1). However, within 8 h, significant mast cell-mediated lysis of all three targets was observed, and by 16 h, maximal mast cell killing had occurred (Fig 1).

Conjugate Formation of Mast Cells With Tumor Targets Although binding of effector cells to tumor targets has proven to be a critical event for some cytotoxic cell populations [30–32], the ability of mast cells to form conjugates with tumors has not been investigated. Therefore, mast cells were cocultured for 16 h with the lytic-sensitive targets, WEHI-164, 5C25, and Currie, and conjugate formation was assessed. Within 1 h of coculture, mast cell binding to the targets was observed, and by 8 h, maximal mast cell conjugate formation with WEHI-164, 5C25, and Currie (23.2 ± 2.1 SEM, 10.2% ± 1.4 SEM, and 10.9% ± 3.5 SEM, respectively) had occurred. Frequently, one or more mast cells was seen surrounding individual tumor cells (Fig 2), and on occasion one mast cell formed conjugates with two or more targets. As a control, mast cells also were cocultured with the lytic-resistant target, YAC-1. Within 1 h, mast cell-YAC-1 conjugate formation was observed and by 8 h maximal binding (16.4% ± 2.3 SEM) had occured. Thus, these experiments indicated that mast cells were capable of binding to tumor targets; however, formation of conjugates with tumor cells alone was not sufficient for cytotoxicity.

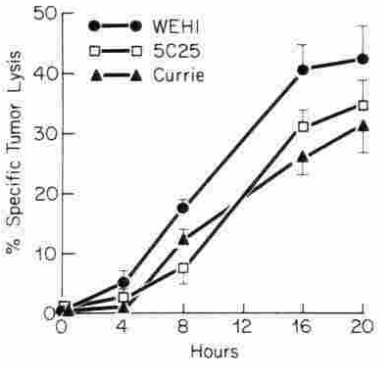

Figure 1. Kinetics of mast cell-mediated cytotoxicity against lytic-sensitive targets. Mast cells were cocultured with WEHI-164, 5C25, and Currie targets at an E:T of 80:1 as described. The data represent the mean and standard error of the mean of three different experiments for each target.

The Potential Role of Mast Cell Granules in Cytotoxicity Because granules from other cytotoxic cell populations have been shown to be important in tumor cell killing [33,34], purified preparations of mast cell granules (MCG) at two different concentrations were investigated for their ability to directly mediate cytotoxicity against WEHI-164, 5C25, and Currie. As seen in Table II, both concentrations of MCG induced significant lysis of WEHI-164 and 5C25 after 16 h (maximal killing of 36.5% ± 6.8 SEM and 34.4% ± 6.9 SEM, respectively). In parallel experiments, however, MCG induced only low levels of cytotoxicity against the Currie tumor (Table II).

Because mast cell killing of Currie appeared to be granule-independent, the potential role of the mast cell plasma membrane in this tumoricidal process also was explored. Mast cell cytoplasts, metabolically active but devoid of nuclei and cytoplasmic organelles [29], were cocultured with Currie tumor cells for 16 h and compared with whole mast cell controls. While whole mast cells induced significant Currie lysis (33.5% ± 7.1 SEM), MCC had no tumoricidal effect. In additional studies not shown, MCC also were not cytotoxic against WEHI-164 or 5C25 tumor cells.

Potential Role of TNF-α in Mast Cell-Mediated Cytotoxicity Previous reports have shown that WEHI-164 cells are lysed by TNF-α, and recent studies have indicated that IL-3-stimulated MMC and basophilic cell lines generate a molecule similar to TNF-α [35–37]. Therefore, the possibility that CTMC-mediated cytotoxicity might be related to a cytokine similar to TNF-α was investigated. WEHI-164, 5C25, and Currie tumor cells were cocultured with different r-TNF-α concentrations (20–20,000 U/ml) and cytotoxicity was determined after 16 h. While WEHI-164 cells were readily killed by low concentrations (20

Table I. Sensitivity of Different Tumor Cell Lines to Mast Cell-Mediated Cytotoxicity

	% Specific Cytotoxicity (± SEM)[a] E:T		
Tumor Cell Line	10:1	40:1	80:1
WEHI-164	19.1 ± 2.9	43.0 ± 2.8	56.0 ± 2.1 (9)[b]
5C25	5.8 ± 3.0	26.1 ± 4.7	34.7 ± 3.4 (7)
Currie	7.1 ± 1.4	18.0 ± 1.1	26.8 ± 2.0 (6)
YAC-1		0.7 ± 0.5	0.6 ± 0.5 (9)
P815		0.3 ± 1.1	1.3 ± 0.9 (3)
K562		2.0 ± 2.1	4.7 ± 3.3 (3)
HEp-2		−0.4 ± 1.5	4.6 ± 1.4 (3)

[a] 16-h cytotoxicity assay.
[b] numbers in parentheses = number of different experiments.

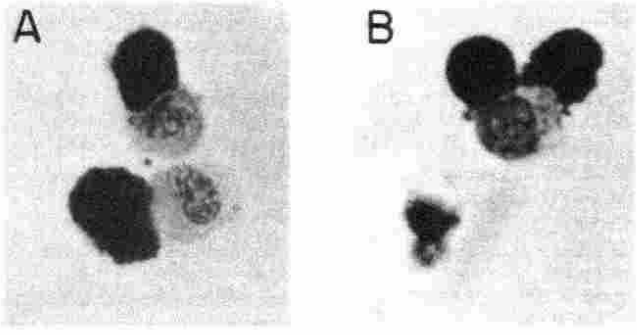

Figure 2. Mast cells *(dark cells)* are bound to WEHI-164 *(A)* and 5C25 *(B)* tumors after 8 h of coculture.

Table II. Mast Cell Granules-Mediate Cytotoxicity

Tumor Cell Line	E:T[a]	% Specific Cytotoxicity (± SEM)[b]
WEHI-164	20:1	17.9 ± 4.2
	40:1	36.5 ± 6.8
5C25	20:1	22.1 ± 4.5
	40:1	34.4 ± 6.9
Currie	20:1	2.5 ± 1.6
	40:1	5.7 ± 2.9

[a] Whole mast cell E:T equivalents.
[b] n = 3 experiments for each condition.

U/ml) of r-TNF-α, 5C25 cells were only slightly TNF-sensitive, and Currie tumor cells were completely resistent to this cytokine (Fig 3). The potential role for a TNF-like molecule in rat CTMC-mediated killing was investigated by coculturing WEHI-164 targets with mast cells (E:T; 40:1) or r-TNF-α (200 U/ml) in the presence of different concentrations (60 NU to 1200 NU) of anti-TNF antibodies. In comparison to control medium or normal rabbit serum, anti-TNF antibodies completely blocked r-TNF-α-induced cytotoxicity and reduced CTMC-mediated killing by nearly 75% (Fig 4). The effect of anti-TNF antibodies on mast cell killing of the slightly TNF-sensitive 5C25 tumor cells also was investigated. As was observed in earlier experiments, 200 U/ml of r-TNF-α induced only low levels of 5C25 lysis, which were completely aborgated by preincubation with 600 NU of anti-TNF antibodies (Fig 5). When 5C25 cells were cocultured with mast cells in the presence of antibodies to TNF, only partial inhibition (approximately 25%) of mast cell-mediated cytotoxicity was observed (Fig 5). In experiments not shown, anti-TNF antibodies (600–2400 NU) had no inhibitory effect on CTMC killing of TNF-resistant Currie tumor cells. Thus, these findings indicated that CTMC induced tumor killing through both a TNF-like factor and a TNF-independent mechanism.

DISCUSSION

The results of this study demonstrate that freshly isolated, rat CTMC express spontaneous cytotoxicity in vitro against specific murine and human tumor cell lines but induce minimal killing of normal cultured human skin fibroblasts or keratinocytes. Kinetic studies revealed that CTMC were capable of forming conjugates with different tumor cells within 1 h of coculture that was maximal by 8 h. It is of interest that the results from these binding experiments paralleled our findings of detectable, significant CTMC-mediated cytotoxicity within 8 h. While our experiments clearly show for the first time that CTMC are capable of binding to tumor cells,

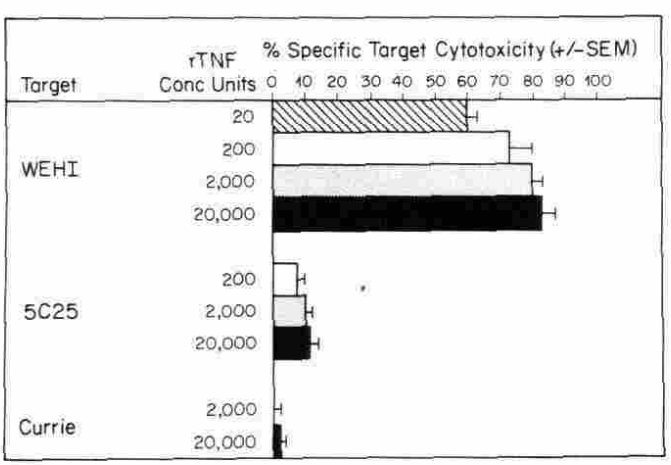

Figure 3. Effect of r-TNF-α on mast cell sensitive targets. WEHI-164, 5C25, and Currie tumor cells were coincubated with different concentrations of TNF. Cytotoxicity was determined as described. The data are the mean and standard error of the mean (SEM) of four experiments for each condition.

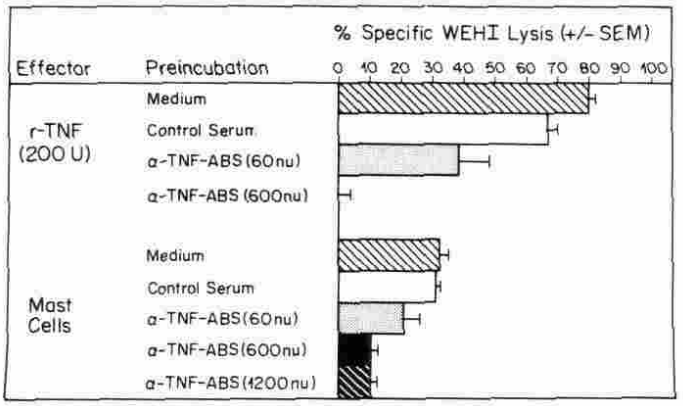

Figure 4. Effect of antibodies to TNF-α (α-TNF-ABS) on TNF and mast cell-mediated lysis of WEHI-164. TNF or mast cells were preincubated for 1 h at 37°C with medium alone, undiluted control rabbit serum, or different concentrations (neutralizing units, nu) of α-TNF-ABS before coculture with WEHI-164 cells. Cytotoxicity was determined as described in *Materials and Methods*. The data are the mean and standard error of the mean (SEM) of three different experiments.

the specificity and importance of this conjugate formation remain in question because CTMC also bind to lytic-resistant YAC-1 cells. We conclude from these observations that the binding of CTMC to tumor cells alone is insufficient for cytotoxicity.

In the last several years, at least two general categories of spontaneous cytotoxic cell populations have been identified and are termed natural killer (NK) cells and natural cytotoxic cells (NC) [38–44]. Although both antitumor populations appear to exist in rodents as well as humans, each has been most extensively characterized in animal systems. Morphologically, NK cells often appear as large granular lymphocytes and are capable of lysing the YAC-1 tumor cell line within 4 h. Rodent NC cells, on the other hand, have not been well-defined morphologically, and may represent more than one population. Nevertheless, cells with NC activity spontaneously kill WEHI-164, but not YAC-1, after 18–20 h of coculture [38–44]. Young and coworkers [36] recently have reported that IL-3 stimulated, immature-appearing mouse mast cell lines lyse both WEHI-164 and YAC-1 tumor cells in vitro after 18 h of coculture. In this same report, partially purified peritoneal mast cells cultured in the presence of IL-3 also were shown to be cytotoxic against both of these tumor targets. These findings are in contrast to reports that have demonstrated that IL-3 stimulated mouse MMC readily kill WEHI-164, but not YAC-1 tumors, in

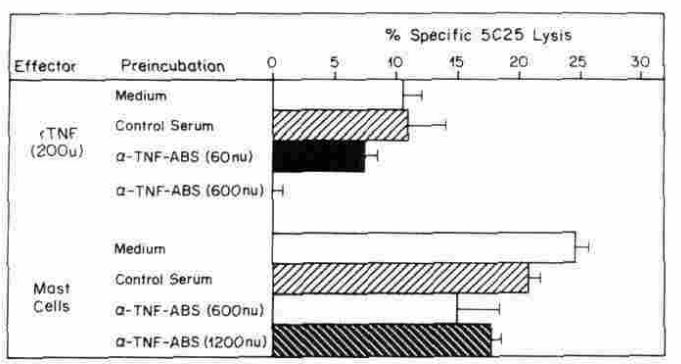

Figure 5. Effect of antibodies to TNF-α (α-TNF-ABS) on TNF and mast cell-mediated lysis of 5C25. TNF or mast cells were preincubated for 1 h at 37°C with medium alone, undiluted control rabbit serum, or different concentrations (neutralizing units, nu) of α-TNF-ABS before coculture with 5C25 cells. Cytotoxicity was determined as described in *Materials and Methods*. The data are the mean and standard error of the mean (SEM) of three different experiments.

vitro within 18 h [19,37,45]. Although the reason for these differing results is unresolved, it may pertain to the homogeneity of the effector cell populations. Our results coincide with the latter studies in that homogenous populations of CTMC are similar to cultured MMC in terms of their cytolytic kinetics and ability to lyse WEHI-164 but not YAC-1 cells. However, an important finding in our study is that CTMC do not require in vitro IL-3 stimulation to mediate cytotoxicity.

Early in our investigation it became evident that CTMC were able to mediate cytotoxicity by a MCG-related mechanism. When lytic-sensitive WEHI-164 and 5C25 targets were cocultured with either intact CTMC or purified MCG, both induced significant tumor cell lysis. In contrast, only CTMC were able to kill Currie tumor cells, suggesting the presence of a second, MCG-independent lytic mechanism. Mast cell cytoplasts were investigated as a potential model for this additional cytotoxic effector system. Although MCC lack nuclei and cytoplasmic structures, they are known to synthesize adenosine triphosphate, incorporate labeled fatty acids into complex lipids, and retain fluorescein after deacylation of diacetylfluorescein [29]. Despite evidence of retained metabolic activities, MCC failed to induce significant killing of the MCG-resistant Currie tumor cells. Thus, we conclude from these observations that MCG are an important mechanism in CTMC-mediated cytotoxicity, whereas the mast cell plasma membrane alone is incapable of inducing significant tumor lysis.

Our investigation of potential mechanisms in CTMC-mediated cytotoxicity focused on the presence of a TNF-like cytokine. This was based on the observations that WEHI-164 tumors could be lysed by TNF-α [35] and that mouse MMC and basophilic cell lines cultured with IL-3 had been reported to generate a molecule similar to TNF [36,37]. Coincubation of the three mast cell-sensitive tumor lines with r-TNF-α confirmed the extreme sensitivity of WEHI-164 to this cytokine; however, 5C25 cells were relatively insensitive, and Currie tumors were very resistant to high TNF concentrations. In additional experiments, antibodies to TNF greatly reduced r-TNF-α and CTMC lysis of WEHI-164, but only partially inhibited killing of 5C25. Taken together, these results indicate that CTMC cytolysis of WEHI-164 occurs predominantly by a TNF-like factor, whereas killing of 5C25 by CTMC is only partially TNF-dependent. The fact that MCG also lyse WEHI-164 and 5C25 suggests that this TNF-like cytokine may be MCG-associated. Our preliminary studies support this idea because we have found that anti-TNF antibodies also inhibit MCG-killing of WEHI-164 and 5C25, and the magnitude of this inhibition is very similar to that observed for intact CTMC. Because our results showed that 5C25 and Currie cells were relatively resistant to TNF, it appears that CTMC-mediated lysis of these two tumors occurs predominantly by a TNF-independent mechanism. Furthermore, the observation that 5C25 cells were lysed by MCG, whereas Currie tumors were MCG-resistant, strongly suggests that CTMC have two non-TNF, cytotoxic mechanisms. Our finding of a TNF-independent killing mechanism(s) in mast cells coincides with the observations of Young and coworkers who reported that anti-TNF antibodies blocked IL-3 stimulated mast cell killing of WEHI-164 but failed to inhibit lysis of TNF-resistant tumor cells. We conclude from our study that CTMC are capable of killing tumors by at least three mechanisms. Lysis of WEHI-164 cells by CTMC appears to occur predominantely through a MCG-associated, TNF-like cytokine, whereas CTMC killing of 5C25 is MCG-related but mostly TNF-independent. The presence of a third cytolytic mechanism is strongly suggested by the finding that CTMC kill Currie targets that are resistant to both MCG and TNF-α.

Although the non-TNF, cytotoxic mechanisms of CTMC remain undefined, several different antitumor effector systems have been characterized in other killer cell populations. These include proteolytic enzymes [46,47], oxygen intermediates, [48,49], ring and tubular structures (perforins and cytolysins) [33,34], and other cytokines [50,51]. Henderson and coworkers [21] have suggested a role for the granule-associated enzyme, peroxidase, as a potential mechanism in mast cell-mediated cytotoxicity. In the presence of added H_2O_2 and iodide, rat mast cells and MCG were shown to kill the lymphoma cell line LSTRA within 60 min, but in the absence of added H_2O_2 or iodide no cytolytic activity was detectable. Although it is conceivable that peroxidase may play some role in CTMC-mediated cytotoxicity, neither H_2O_2 nor iodide was included in our experimental conditions, and significant killing of tumor cells was not observed until 8 h. In studies not shown, we also have investigated the preformed MCG-associated mediators, histamine and heparin, for their potential cytotoxic effects against the three CTMC-sensitive targets; however, over broad concentration ranges, neither histamine (10^{-9}–10^{-3} M) nor heparin (1 ng/ml–1 mg/ml) alone or in combination induced tumor cell killing. Recent studies also have demonstrated that cell lines with NK activities release small tubular complexes (perforins and cytolysins) that insert into target cell membranes and form pores that lead to tumor lysis [33,34]. Although our present studies show that CTMC killing of WEHI-164 and 5C25 is MCG-related, preliminary electron microscopy studies of mast cell-tumor target cocultures have not demonstrated the presence of these pore-forming structures. The investigation of other non-TNF, CTMC-mediated cytotoxic mechanisms is currently being pursued.

Several lines of evidence suggest an important in vivo role for CTMC in tumor surveillance. Studies performed in mice bearing experimentally induced tumors indicate that stimulation of mast cells that surround subcutaneous fibrosarcomas leads to a significant decrease in tumor size [8]. In addition, an inverse correlation between skin mast cell content and the incidence of transplanted fibrosarcomas in the skin of mast cell deficient (W/W^v) and normal congenic mice has been observed [6,7]. An in vivo role for human skin mast cells in tumor surveillance also has been suggested by studies reporting an increase in CTMC numbers within and surrounding some neoplasms invading the dermis [9,10]. In preliminary work from our laboratory, human, dermal mast cells have been quantified in histologic sections of basal cell carcinomas (BCC) and squamous cell carcinomas (SCC) using a morphometric point counting technique [52]. Our initial findings indicate that BCC and SCC have a ten-fold and seven-fold increase, respectively, in dermal mast cell content. Thus taken together, these observations suggest that CTMC may play a primary role in response to neoplasms invading the dermis.

The results of this in vitro study indicate that CTMC represent a distinct antitumor cell population with some of the functional characteristics associated with NC cell and MMC-mediated cytotoxicity; however, CTMC appear to have more diverse cytotoxic mechanisms than these other antitumor populations. Studies are now in progress to further define the mechanisms by which CTMC mediate these tumoricidal effects and to investigate the potential role of CTMC in defense against cutaneous neoplasms.

The authors wish to thank Mrs. Kathy Lober for her expert technical assistance and Mrs. Mary Beth Bawden for her excellent preparation of the manuscript. We also wish to thank Dr. Leo S. Lin, Cetus Corporation, for his support of our work.

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