The immunorerestorative effect of Cimetidine in vitro on the T cell-induced local GVH reaction in vivo was studied in 43 cancer patients and 43 normal healthy donors. Both low dose (10^{-5} M) and high dose (10^{-4} M) Cimetidine induced significant, albeit partial, immune restoration among GVHR-negative cancer patients (p < 0.05, p < 0.01, respectively) with the high dose being significantly more effective (p < 0.05). In contrast, similar Cimetidine doses induced only moderate augmentation (p > 0.05) among GVHR-positive cancer patients and a marginal one among normal healthy donors. In the latter 2 groups, Cimetidine was found to be occasionally detrimental in that it induced a conversion from a positive to a negative GVH reaction.

These results support the concept of anti-suppressor cell activity ascribed to Cimetidine. However, the possibility of a detrimental effect should be born in mind in planning future clinical trials. We propose that the use of Cimetidine be limited to cancer patients with documented increase in suppressor cell activity associated with defective T cell function under close serial monitoring.

A growing body of animal and human data now tends to focus on the suppressor T cells as a possible pathologic regulatory component involved in the development and the course of malignant and nonmalignant disease processes (1–3). Human suppressor T cells are characterized by surface receptors to the Fc fraction of IgG and by receptors to histamine (4–7). The role of these receptors in the mechanism of suppression is yet to be determined. However, histamine was found to exert an immunosuppressive effect (8, 9) probably through release of soluble suppressive factors from these cells—an effect that could be blocked by H2-receptor antagonist. (5)

Theoretically, H2-receptor antagonists may be used as therapeutic immunomodulators intended to inhibit pharmacologically T suppressor cells. Cimetidine, an H2-receptor antagonist was shown to augment delayed-type hypersensitivity reactions in vivo and lymphocyte blastogenesis in vitro among human subjects (10–12). Using an immunobiosay, the local xenogeneic graft-vs-host reaction (GVHR) as a practical tool for the assessment of T cell function (13) we also demonstrated a monocyte-dependent restoration of the local GVHR among cells from cancer patients that was pharmacologically induced by Indomethacin (14). In this report we wish to present our experience with the effect of Cimetidine in vitro on the local GVHR among mononuclear cells (MNC) from cancer patients and from normal donors.

MATERIALS AND METHODS

MNC were isolated from heparinized venous blood of 43 patients with disseminated cancer and from 43 normal, healthy individuals as previously described. (15). The patients included 12 with lung carcinoma, 9 with squamous cell carcinoma of the head and neck, 4 breast cancer, 4 with sarcoma, 4 with malignant melanoma, 3 with adenocarcinoma of unknown primary, 2 with Hodgkin’s disease, and 1 each with breast carcinoma and chronic lymphocytic leukemia. All patients were either previously untreated or off all treatments ≥ 4 wk before study. The MNC were resuspended in RPMI 1640 (Grand Island Biological Company, Grand Island, NY) supplemented with 10% fetal calf serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin. Aliquots of MNC were incubated with either 10^{-5} M or 10^{-4} M Cimetidine or left untreated (controls). All cells were inoculated at 37°C for 30 min in a humidified atmosphere containing 5% CO2. After incubation, the cells were washed 3 times and reconstituted with RPMI 1640 to a volume of 0.2 ml containing 2 x 10^9 MNC. Cimetidine-treated and untreated cells from cancer patients and from normal healthy individuals were injected intradermally into partially immunosuppressed rats for the local GVHR reaction as previously described. (13) The volume of the local GVHR reaction was assessed 48 hr later as previously described. (13) GVH nodules of <50 mm^3 were defined as ‘negative GVHR reactions’ and were indicative of T cell dysfunction. GVH nodules ≥ 50 mm^3 were considered ‘positive GVHR reactions’ and were indicative of T cell competence. To assess the effect of Cimetidine on the local GVHR reactions, the percent change induced by cimetidine was calculated as follows:

$$\% \text{GVHR change} = \left( \frac{\text{Cimetidine} - \text{no Cimetidine}}{\text{No Cimetidine}} \right) \times 100.$$  

A conversion of a negative GVHR reaction (<50 mm^3) to a positive one (>50 mm^3) or an increase of 50% or more in the GVHR reaction was defined as immunorestoration or immunoaugmentation, respectively. Immunodepression was defined as a decrease of 50% or more in the GVHR reaction. Statistical analysis of changes in GVHR reaction volumes was performed by the Wilcoxon sign-rank test for paired data and by the Wilcoxon-Mann-Whitney test for unpaired data. (16) The incidence of immune restoration or immune augmentation was analyzed by the χ^2 test.

RESULTS

The in vitro effect of Cimetidine on the GVHR reaction produced by MNC from 43 cancer patients and 43 normal donors is shown in Table I. Twenty-six of the 43 cancer patients were initially found to be GVHR negative (<50 mm^3) and 17 were GVHR positive. Forty-one of the normal donors were also GVHR positive initially and 2 were negative. GVHR restoration was achieved in 6 of 11 GVHR negative cancer patients whose MNC were incubated with 10^{-5} M Cimetidine. The median GVHR volume produced by untreated MNC was 15 mm^3 whereas that which was produced by the Cimetidine-treated MNC was 25 mm^3. This increase in the GVHR volume was statistically significant (p < 0.05). With a higher Cimetidine concentration (10^{-4} M) 14 of 15 GVHR-negative cancer patients showed immune restoration. The median GVHR volume produced by untreated MNC was 17 mm^3 whereas that which was produced by Cimetidine-treated (10^{-4} M) MNC was 85 mm^3 (p < 0.01). It is noteworthy that the higher concentration was more effective than...
the lower concentration of Cimetidine in restoring the local GVH reaction \( (p < 0.05) \) both in terms of the incidence of conversion from negative to positive \( (14 of 15 vs 6 of 11) \) and the median volume of the reaction \( (25 mm^2 vs 85 mm^2) \).

Local GVHR augmentation was noted in 3 of 9 GVH-positive cancer patients whose MNC were incubated with \( 10^{-5} \) M Cimetidine. The median GVHR volume produced by untreated MNC was 73 mm^2 whereas that which was produced by the Cimetidine-treated MNC was 110 mm^2 \( (p > 0.05) \). With the higher Cimetidine concentration \( (10^{-4} M) \) we also noted GVHR augmentation in 5 of 8 GVH-positive cancer patients with a median GVHR volume of 121 mm^2 for untreated MNC compared to a median of 139 mm^2 for Cimetidine-treated MNC \( (p > 0.05) \). However, in contrast to the observation among GVHR-negative cancer patients, the high concentration of Cimetidine was not more effective than the low concentration in restoration among GVHR-positive and GVHR-negative patients whose GVHR converted from positive to negative after incubation of their MNC with Cimetidine.

The incidence of MNC from normal donors with either high or low concentration of Cimetidine \textit{in vitro} also produced insignificant differences in the local GVHR reaction. The effect was mixed, and augmentation in some cases was offset by suppressive effect in others.

**DISCUSSION**

The results of this study emphasize the scope of immune modulation exerted by Cimetidine \textit{in vitro} on the local GVH reaction. The restorative effect of Cimetidine was most pronounced among cancer patients. Those patients who were initially characterized by defective T cell function as manifested by negative local GVH reaction. Since biopsies of such negative local GVH reaction sites in the rats' skin were found to contain suppressor cells. Incubation of these potential suppressor cells with Cimetidine would therefore abrogate their activation by the histamine and result in restoration of the local GVH reaction. The restorative effect in this group of patients was greater when a higher concentration of Cimetidine was used. This may raise the possibility of nonspecific histamine-induced suppressor cells, it now appears that the concanavalin A-activated suppressor T cells also belong to the histamine receptor-bearing population of lymphocytes. (18) Furthermore, the activation of suppressor T cells by concanavalin A seems to be mediated through histamine release \( (19) \) and/or perhaps the enhanced expression of histamine receptor since it can be blocked by either histamine or by Cimetidine. (19, 20)

In addition to the naturally occurring histamine receptor-bearing suppressor T cells, it now appears that the concanavalin A-activated suppressor T cells also belong to the histamine receptor-bearing population of lymphocytes. (18) Furthermore, the activation of suppressor T cells by concanavalin A seems to be mediated through histamine release \( (19) \) and/or perhaps the enhanced expression of histamine receptor since it can be blocked by either histamine or by Cimetidine. (19, 20)

**TABLE I**

<table>
<thead>
<tr>
<th>Cimetidine Concentration</th>
<th>No. Restored or Augmented No. Studied</th>
<th>GVH Volume in mm$^2$ Median (Range)</th>
<th>No Cimetidine</th>
<th>Cimetidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GVH negative</td>
<td>$10^{-4} M$ 6/11$^*$</td>
<td>15 (0–36)$^*$</td>
<td>25 (0–62)$^*$</td>
<td></td>
</tr>
<tr>
<td>GVH positive</td>
<td>$10^{-4} M$ 14/15$^*$</td>
<td>17 (0–44)$^*$</td>
<td>85 (7–1014)$^*$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^{-3} M$ 3/9$^*$</td>
<td>73 (52–122)$^*$</td>
<td>110 (61–163)$^*$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^{-2} M$ 5/8$^*$</td>
<td>121 (51–138)$^*$</td>
<td>139 (29–2532)$^*$</td>
<td></td>
</tr>
<tr>
<td>Normal donors</td>
<td>$10^{-4} M$ 4/18$^*$</td>
<td>85 (36–838)$^*$</td>
<td>104 (19–537)$^*$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^{-3} M$ 5/25$^*$</td>
<td>95 (29–294)$^*$</td>
<td>105 (5–377)$^*$</td>
<td></td>
</tr>
</tbody>
</table>

* $vs.$ $p < 0.05.$  
* $vs.$ $p = 0.05.$  
* $vs.$ $p > 0.01.$  
* $vs.$ $p < 0.05.$  
* $vs.$ $p = 0.05.$  
* $vs.$ $p > 0.05.$  
* All but one normal donor were GVH-positive.

REFERENCES


