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IMMUNE RESTORATION AND/OR AUGMENTATION OF LOCAL XENOGENEIC GRAFT *VERSUS* HOST REACTION BY CIMETIDINE *IN VITRO*[1]

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The immunorestorative 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 H_2-receptor antagonist. (5)

Theoretically, H_2-receptor antagonists may be used as therapeutic immunomodulators intended to inhibit pharmacologically T suppressor cells. Cimetidine, an H_2-receptor antagonist was shown to augment delayed-type hypersensitivity reactions *in vivo* and lymphocyte blastogenesis *in vitro* among human subjects (10–12). Using an immunobioassay, the local xenogeneic graft-*vs*-host reaction (GVHR)[3] 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, 7 with colorectal 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 incubated at 37°C for 30 min in a humidified atmosphere containing 5% CO_2. After incubation, the cells were washed 3 times and reconstituted with RPMI 1640 to a volume of 0.2 ml containing 2×10^7 MNC. Cimetidine-treated and untreated cells from cancer patients and from normal healthy individuals were injected intradermally into partially immunosuppressed rats for the local GVH reaction as previously described. (13) The volume of the local GVH reaction was assessed 48 hr later as previously described. (13) GVH nodules of <50 mm³ were defined as "negative GVH reactions" and were indicative of T cell dysfunction. GVH nodules ≥ than 50 mm³ were considered "positive GVH reactions" and were indicative of T cell competence. To assess the effect of Cimetidine on the local GVH 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 GVH reaction (<50 mm³) to a positive one (>50 mm³) or an increase of 50% or more in the GVH reaction were defined as immunorestoration or immunoaugmentation, respectively. Immunodepression was defined as a decrease of 50% or more in the GVH reaction. Statistical analysis of changes in GVH 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 GVH 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 GVH negative (<50 mm³) and 17 were GVH positive. Forty-one of the normal donors were also GVH positive initially and 2 were negative. GVHR restoration was achieved in 6 of 11 GVH negative cancer patients whose MNC were incubated with 10^{-5} M Cimetidine. The median GVHR volume produced by untreated MNC was 15 mm³ whereas that which was produced by the Cimetidine-treated MNC was 25 mm³. This increase in the GVH volume was statistically significant ($p < 0.05$). With a higher Cimetidine concentration (10^{-4} M) 14 of 15 GVH-negative cancer patients showed immune restoration. The median GVH volume produced by untreated MNC was 17 mm³ whereas that which was produced by Cimetidine-treated (10^{-4} M) MNC was 85 mm³ ($p < 0.01$). It is noteworthy that the higher concentration was more effective than

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[3] Abbreviations used in this paper: GVHR, graft-*vs*-host reaction; MNC, mononuclear cells

TABLE I

Effect of Cimetidine in vitro on local GVH reaction among cancer patients and normal donors

	Cimetidine Concentration	No. Restored or Augmented/No. Studied	GVH Volume in mm^3 Median (Range)	
			No Cimetidine	Cimetidine
Cancer patients				
GVH negative	10^{-5} M	6/11[e]	15 (0–36)[a]	25 (0–62)[b]
	10^{-4} M	14/15[f]	17 (0–44)[c]	85 (7–1014)[d]
GVH positive	10^{-5} M	3/9	73 (52–122)[g]	110 (81–163)[h]
	10^{-4} M	5/8	121 (51–138)[i]	139 (29–2532)[i]
Normal donors	10^{-5} M	4/18[k]	85 (36–838)	104 (19–537)
	10^{-4} M	5/25[k]	95 (29–294)	105 (5–377)

[a] *vs* [b] $\approx p < 0.05$.
[b] *vs* [d] $\approx p < 0.05$.
[c] *vs* [d] $\approx p < 0.01$.
[e] *vs* [f] $\approx p < 0.05$.
[g] *vs* [h] $\approx p > 0.05$.
[i] *vs* [i] $\approx p > 0.05$.
[k] All but one normal donor were GVH-positive.

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^3 *vs* 85 mm^3).

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^3 whereas that which was produced by the Cimetidine-treated MNC was 110 mm^3 ($p > 0.05$). 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^3 for untreated MNC compared to a median of 139 mm^3 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 among GVHR-positive patients and was even suppressive in 1 patient whose GVHR converted from positive to negative after incubation of his MNC with Cimetidine.

The incubation of MNC from normal donors with either high or low concentration of Cimetidine *in vitro* also produced insignificant differences in the local GVH 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 *in vitro* on the local GVH reaction. The restorative effect of Cimetidine was most pronounced among cancer 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 have been characterized by abundant infiltration with basophils (13), it is conceivable that these cells may be the source of histamine release, which in turn activates the histamine receptor-bearing suppressor cells. Incubation of these potential suppressor cells with Cimetidine *in vitro* before grafting into the rats' skin may 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 (rather than H_2-antagonist) drug effect among T cell-function-deficient cancer patients. Using levamisole, a structurally related (imidazole ring) immunomodulating agent in similar patients, no restoration of the local GVHR was noted after incubation *in vitro*. (Mavligit G.: unpublished data).

The augmentative effect of Cimetidine among cancer patients who were initially characterized by T cell competence, as indicated by positive local GVH reaction, was less pronounced. Moreover, the higher concentration of Cimetidine not only failed to effect further augmentation in this group, but was even detrimental in 1 case. The latter phenomenon was even more common among our normal healthy donors. It is noteworthy that such detrimental effect was not reported by other investigators who studied the *in vitro* effect of Cimetidine on lymphocyte blastogenesis. (12) This suggests that under certain circumstances, the effect of Cimetidine *in vitro* could be of dual nature. A case can therefore be made to limit the future experimental use of Cimetidine as a potential immunorestorative agent *in vivo* to a selective group of patients who are characterized by a profound T cell dysfunction. (11, 17)

A growing body of evidence now exists to suggest that histamine (H_2-type) receptor-carrying suppressor T cells may play an important immunoregulatory role in the execution of the normal immune response whereas aberrations (excess numbers or the lack thereof) in this subpopulation are often shown to be associated with the development of pathologic conditions. (5–7) Thus, it is currently believed that the suppressive effect of these suppressor T cells is mediated by the release of a soluble factor induced by histamine since it can be abrogated by Cimetidine. (5)

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 histaminase or by Cimetidine. (19, 20)

The demonstration of histamine-related anti-suppressor cell effect of Cimetidine has led to a therapeutic Phase I trial with this drug as an augmentative biologic response modifier among immune incompetent cancer patients with increased suppressor T cell activity as recently shown in our laboratory. (21) The clinical trial is conducted with a careful monitoring of both immunologic parameters and the possible clinical side effects that may be anticipated in connection with the use of Cimetidine *in vivo*, as was extensively reported in various gastrointestinal disorders. (22) It is hoped that the anti-suppressor T cell activity of Cimetidine will outweigh these side effects and result in a favorable effect on the clinical course of the neoplastic disease.

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