Cimetidine modulates the antigen presenting capacity of dendritic cells from colorectal cancer patients

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Cimetidine, a histamine type 2 (H2) receptor antagonist, has been reported to improve survival in gastrointestinal cancer patients. These effects have largely been attributed to the enhancing effects of cimetidine on the host's antitumour cell-mediated immune response, such as inhibition of suppressor T lymphocyte activity, stimulation of natural killer cell activity and increase of interleukin-2 production from helper T lymphocytes. We conducted an in vitro study on the effects of cimetidine on differentiation and antigen presenting capacity of monocyte-derived dendritic cells from advanced colorectal cancer patients and normal controls. As a result, an investigation of expression of surface molecules associated with dendritic cells by flow cytometric analyses showed that cimetidine had no enhancing effect on differentiation of dendritic cells from cancer patients and normal controls. An investigation of [3H]thymidine incorporation by allogeneic mixed lymphocyte reactions revealed that cimetidine increased the antigen presenting capacity of dendritic cells from both materials. Moreover, a higher antigen presenting capacity was observed in advanced cancer patients compared to normal controls. These effects might be mediated via specific action of cimetidine and not via H2 receptors because famotidine did not show similar effects. Our results suggest that cimetidine may enhance the host's antitumour cell-mediated immunity by improving the suppressed dendritic cells function of advanced cancer patients.

British Journal of Cancer (2002) 86, 1257 – 1261. DOI: 10.1038/sj/bjc/6600233 www.bjcancer.com
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Keywords: dendritic cell; H2 receptor antagonist; cimetidine; colorectal cancer

MATERIALS AND METHODS

Patients and controls

The study has been carried out with the ethical committee approval. Ten patients (four men and six women) with advanced...
colorectal cancer, aged 28–65 years (means ± s.d.; 50.6 ± 11.5 years) were studied (Table 1). All tumours were classified as stage IV according to tumour-node-metastasis (TNM). All patients had received chemotherapy and/or operation and had the interval of more than 4 weeks prior to the present study. Their leukocyte numbers were within normal limits. The control subjects consisted of 10 age-matched healthy volunteers (eight men and two women). All patients and all healthy volunteers were free from infection and other complications at the time of study.

### Media and reagents

RPMI 1640 supplemented with 4 mM l-glutamine and NaHCO\(_3\) (Nikken, Kyoto, Japan), 100 IU ml\(^{-1}\) penicillin and 100 μg ml\(^{-1}\) streptomycin (Sigma, UK), 50 μM 2-mercaptoethanol, and 10% heat-inactivated foetal calf serum (FCS) was used as culture medium throughout the experiments. Human recombinant granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) were kindly provided by Kirin Brewery (Gunma, Japan) and Genzyme (Minneapolis, MN, USA), respectively. Cimetidine and famotidine were kindly provided by Smith Kline Beecham, Japan (Tokyo, Japan) and Yamanouchi (Tokyo, Japan), respectively.

### Generation of DC

PBMC were obtained from 10 patients with advanced colorectal cancer by leukapheresis using Blood Cell Separator CS-3000\(^{TM}\) (Baxter, Deerfield, IL, USA) after informed consent was obtained. As control subjects, PBMC from healthy volunteers were prepared by density gradient centrifugation on Ficoll-Hypaque Plus (Pharmacia Biotech, Sweden). Interphases were harvested and washed twice with RPMI 1640 at low speed to remove platelets. Monocytes were separated from these PBMC by plastic dish adhesion for 2 h twice with RPMI 1640 at low speed to remove platelets. Monocytes were isolated from PBMC as described above. Allogeneic T lymphocytes were generated from PBMC of a single healthy volunteer by nylon fibre non-adherence using T lymphocyte isolation columns (Nylon Column T, Wako, Japan). The stimulator cell fractions (DC) were irradiated with 30 Gy. After extensive washing different numbers of stimulators were added to the culture wells containing a fixed amount of T lymphocytes (10\(^5\) well\(^{-1}\)) so that the final stimulator to responder ratio (R/S ratio) ranged from 20/1 to 80/1. At day 0 of coculture, 1.0 μg ml\(^{-1}\) of cimetidine or 0.1 μg ml\(^{-1}\) of famotidine was added to the culture medium. During the last 8 h of 5 days of culture, 1 μCi well\(^{-1}\) [\(^{3}H\)]thymidine (Amersham Pharmacia Biotech, UK) was added. Cells were then harvested and radioactivity of [\(^{3}H\)]thymidine incorporated per well. The stimulation index (S.I) was used to quantify the frequency of allogeneic T lymphocyte proliferation. The S.I was expressed as the ratio of c.p.m. with cimetidine or famotidine to c.p.m. without them.

### IL-12 assay

DC from colorectal cancer patients and healthy volunteers, and allogeneic T lymphocytes from a single healthy volunteer were cocultured at R/S ratio of 10/1 in the presence of 1.0 μg ml\(^{-1}\) of cimetidine. After 5 days of culture, supernatants were centrifuged to remove residual cells and stored in −20°C until use. IL-12 p70 heterodimer levels in the supernatants were measured by sandwich type enzyme-linked immunosorbent assay (ELISA) (Immunotech, France) according to the manufacture’s instructions. All tests were performed in duplicate. The sensitivity levels of the ELISA assays were 5 pg ml\(^{-1}\).

### Statistical analysis

Results were presented as means ± standard deviation (s.d.). Student’s t-test was applied to test significant differences and a P value of <0.05 was considered to indicate statistical significance. All tests were two-tailed.

### RESULTS

#### Effect of cimetidine and famotidine on DC differentiation

Flow cytometry was used to investigate the effect of cimetidine and famotidine on the differentiation of DC. Expression of MHC–class II, CD80 and CD86 was analysed and viability of differentiated cells was measured. As a result, no enhancing effect of cimetidine on DC differentiation was found. As shown in Table 2, cimetidine slightly increased the expression of surface molecules only in Case 1, but not in the

### Table 1 Patient characteristics

| Case no. | Age (years old) | Sex | Disease | Clinical stage (metastases) |
|----------|-----------------|-----|---------|---------------------------|
| 1        | 57              | F   | Rectal cancer | IV (lung, adrenal gland) |
| 2        | 28              | F   | Colon cancer  | IV (peritoneum)          |
| 3        | 56              | M   | Rectal cancer  | IV (liver)               |
| 4        | 42              | F   | Colon cancer  | IV (liver)               |
| 5        | 60              | F   | Rectal cancer  | IV (peritoneum)          |
| 6        | 65              | F   | Colon cancer  | IV (liver)               |
| 7        | 53              | M   | Colon cancer  | IV (brain)               |
| 8        | 60              | F   | Colon cancer  | IV (liver)               |
| 9        | 46              | F   | Colon cancer  | IV (ovary, bone)         |
| 10       | 39              | M   | Colon cancer  | IV (lung)                |
other cases tested. The analysis was stopped in Case 6 because positive data were not found after Case 2. Famotidine showed no effects in any cases tested (data not shown). Similar results were obtained in healthy volunteers (data not shown) or at increasing concentrations (5-, 10-, 50-fold) of each H₂ receptor antagonist (data not shown). These results were substantiated by the fact that both cimetidine and famotidine did not enhance the viability of differentiated cells (data not shown).

**Effect of cimetidine and famotidine on antigen presenting capacity of DC**

Allo MLR was carried out to investigate the effect of cimetidine and famotidine on the antigen presenting capacity of DC, and [³H]thymidine incorporation of allogenic T lymphocytes was measured. Monocyte-derived DC generated as described in Materials and Methods were cocultured with allogenic T lymphocytes from a single healthy volunteer in the presence of 1.0 µg ml⁻¹ cimetidine or 0.1 µg ml⁻¹ famotidine.

As a result, in eight out of 10 colorectal cancer patients, cimetidine obviously increased [³H]thymidine incorporation of allogenic T lymphocytes compared to famotidine (Figure 1). In two typical cases (Case 2 and Case 6), cimetidine increased significantly and constantly [³H]thymidine incorporation at each R/S ratio of 20/1 to 80/1 (Figure 2). Moreover, mean S.I of cimetidine at each R/S ratio in all cases was significantly higher than that of famotidine (Figure 3). In a comparison between colorectal cancer patients and normal controls, cimetidine showed higher increases in the former than in the latter (P=0.048 at 20/1) (Figure 4). On the other hand, famotidine did not show any increase both in cancer patients and normal controls (data not shown).

**Effect of cimetidine on IL-12 production of DC**

IL-12 concentrations of the supernatants obtained by coculture of DC with allogeneic T lymphocytes were measured to evaluate the effect of cimetidine on DC function.

IL-12 production of DC in colorectal cancer patients (n=7) was slightly lower than in normal controls (n=4). Although cimetidine did not affect IL-12 production of DC in normal controls, it tended to increase IL-12 production in colorectal cancer patients up to the level of normal controls (P=0.383) (Table 3).

**DISCUSSION**

The clinical effectiveness of cimetidine against gastrointestinal malignancies has been reported and various mechanisms of action have been proposed. In this study, we discovered for the first time the possibility that cimetidine may increase the antigen presenting capacity of monocyte-derived DC from advanced colorectal cancer patients although it does not enhance their differentiation. These results suggest that cimetidine enhances antitumour cell-mediated immune response by stimulating DC to activate Th1 type immune response and subsequent CTL induction. Gifford and Tirberg (1987) demonstrated that cimetidine increased IL-2 production from mitogen-activated murine spleen cells and this effect might be due to stimulation of helper T lymphocytes by antigen presenting cells. The present results support their findings.

Our observation that cimetidine increased the antigen presenting capacity of DC from colorectal cancer patients compared to DC from normal controls implies improvement of suppressed DC function in immunosuppressed cancer patients by cimetidine. Dysfunction of DC in advanced cancer patients is predictable and Ninomiya et al (1999) have demonstrated that DC from hepatocellular carcinoma had significantly lower capacity to stimulate allogeneic T lymphocytes in allo MLR compared to DC from normal controls. The stimulatory effect of cimetidine on T lymphocytes is well-known (Rocklin, 1976; Gifford et al, 1980), however, it is unlikely that the difference of [³H]thymidine incorporation between cancer patients and normal controls is caused by flow cytometry. Data are presented as mean channel ratio as described.

Table 2  Effect of cimetidine on DC differentiation

| Case | C 1.0 | C 10 | C 1.0 | C 10 | C 1.0 | C 10 | Enhancement |
|------|-------|------|-------|------|-------|------|-------------|
| 1    | 1.29  | 1.70 | 1.12  | 1.05 | 1.31  | 2.43 | Yes        |
| 2    | 0.59  | 0.85 | 0.94  | 1.01 | 0.88  | 0.87 | No         |
| 3    | 1.17  | 0.90 | 0.97  | 0.94 | 1.03  | 1.14 | No         |
| 4    | 0.94  | 0.93 | 1.08  | 0.86 | 0.94  | 1.31 | No         |
| 5    | 0.96  | 0.99 | 0.77  | 0.82 | 0.88  | 0.70 | No         |
| 6    | ND    | ND   | ND    | ND   | ND    | ND   | –          |
| 7    | ND    | ND   | ND    | ND   | ND    | ND   | –          |
| 8    | ND    | ND   | ND    | ND   | ND    | ND   | –          |
| 9    | ND    | ND   | ND    | ND   | ND    | ND   | –          |
| 10   | ND    | ND   | ND    | ND   | ND    | ND   | –          |

*C 1.0: Cimetidine 1.0 µg ml⁻¹; C 10: Cimetidine 10 µg ml⁻¹; ND: not done; Mean channel ratio; Mean channel of C 1.0, mean channel of C 0; Mean channel of C 10, mean channel of C 0. The expression of MHCIi Class II, CD80 and CD86 are analysed by flow cytometry. Data are presented as mean channel ratio as described.

**Figure 1**  Antigen presenting capacity of two typical cases (Case 2 and Case 6). Data are presented as amounts (c.p.m.) of [³H]thymidine incorporation.
only by the effect of cimetidine on T lymphocytes because T lymphocytes from a single healthy volunteer were used as responders in allo MLR.

To confirm the hypothesis that cimetidine gives a direct action to DC themselves and improve the antigen presenting capacity of DC from colorectal cancer patients, we measured IL-12 in the supernatants of allo MLR. IL-12 is well known as a cytokine that is produced by DC responding to antigen stimulation and acts on CD4+ helper T lymphocytes to induce Th1-type immune responses (Banchereau and Steinman, 1998). The present results indicate that IL-12 was produced from DC stimulated by allogeneic T lymphocytes and cimetidine might improve the suppressed DC function of colorectal cancer patients. Therefore, we conclude that the increase of [3H]thymidine incorporation in allo MLR may be due to some effects of cimetidine on not only T lymphocytes but also DC themselves or the interaction between DC and T lymphocytes.

On the other hand, famotidine, another H2 receptor antagonist, did not show the same effects as cimetidine. Because famotidine behaves as a specific H2 receptor antagonist with a molar potency four to eight times greater than that of cimetidine (Pedra et al, 1982; Feldman and Burton, 1990), it is

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Table 3  IL-12 production by allo MLR

|            | Cimetidine (µg ml⁻¹) | IL-12 (pg ml⁻¹) |
|------------|----------------------|-----------------|
| Normal controls (n=4) | 0                    | 46.7±1.6        |
|            | 1.0                  | 47.5±3.4        |
| Colorectal cancer patients (n=7) | 0                    | 43.4±1.7        |
|            | 1.0                  | 47.3±3.6        |

Comparison of IL-12 production by allo MLR with colorectal cancer patients and normal controls. Data are presented as means±s.d.
natural that famotidine should show equal or greater effects if the effect of cimetidine is mediated via H2 receptors. In this regard, cimetidine has been reported to have better cell-mediated immunomodulation (e.g., proliferation and cytotoxicity of lymphocytes) or histamine (or H2 receptor) dependent inhibitory effects on tumour growth than other H2 receptor antagonists such as famotidine and ranitidine, and the differences between cimetidine and other H2 receptor antagonists might be due to their structures and/or affinities to H2 receptors (Morris and Adams, 1995; Lawson et al., 1996). Kobayashi et al. (2000) showed that cimetidine can block the adhesion of colorectal cancer cells to the endothelial cells, suppressing the metastases of cancer cells. They also considered that these actions of cimetidine are not mediated via H2 receptors, because other H2 receptor antagonists, famotidine and ranitidine, did not show a similar effect. While it remains unclear whether H2 receptors are expressed on DC or not, the effect of cimetidine on the antigen presenting capacity of DC appears to arise because of cimetidine-specific actions.

Although it remains unclear whether or not the modulating effects of cimetidine on DC function observed in our investigation in vitro have clinically substantial meanings, clinical effectiveness of cimetidine against gastrointestinal malignancies are considered to be due to the total of immunological and non-immunological actions of cimetidine.

Finally, both tumour-antigen-specific and non-specific immunosuppression have been observed in the tumour-bearing host (Roth, 1983; Ninomiya et al., 1999). Therefore, immunostimulation offers theoretical benefits for immunotherapy. Further investigation into DC functions is promising in the search for more clinically effective tumour-antigen-specific immunotherapy and also for the elucidation, of immunosuppressive mechanisms in tumour-bearing hosts.

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