**HLA Type and Survival in Gastric Cancer**

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**ABSTRACT**

We studied HLA typing of 17 stage III gastric cancer patients who survived for more than 5 years after gastrectomy, in comparison with normal healthy controls and other gastric cancer patients. As a result, the long-term survivors had a high frequency of HLA-DR4 antigen compared with normal healthy controls ($P < 0.0025$). This remains statistically significant after correction of the $P$ value according to the number of HLA-DR antigens tested (corrected $P$ value, $<0.025$), strongly suggesting that the presence of this antigen is associated with long-term survival time for postoperative stage III gastric cancer patients.

**INTRODUCTION**

We have shown that nonspecific immunotherapy with BCG-CWS\textsuperscript{2} has a beneficial effect on the survival of postoperative gastric cancer patients (1). Based on a randomized controlled trial by an envelope method after operation, postoperative treatments of the patients were divided into three groups, control, chemotherapy, and chemotherapy plus immunotherapy with BCG-CWS. As a result, the beneficial effect of immunotherapy was noted to be great particularly in advanced stage III and IV (microscopic clinical stage) gastric cancer.

In mouse models, genetic regulation of CML responses to antigenic determinants that are recognized in association with H-2K- and H-2D-coded self-structures have been reported for trinitrophenyl-modified cells (2) and viral-infected cells (3). Furthermore, the strains expressing the $k$ allele in the K region (H-2$^k$ or H-2$^a$) have been found to be high responders to syngeneic trinitrophenyl-modified spleen cells conjugated with a limiting range of concentrations of trinitrobenzene sulfonate compared to H-2$^b$ or H-2$^d$ strains (4). Moreover, in an experiment using C57BL/10 congenic and recombinant mice and their Rous sarcoma virus-induced syngeneic fibrosarcomas, it is found that high responders of the strains expressing H-2D$^d$ are able to induce cytotoxic activities against syngeneic Rous sarcoma virus-induced fibrosarcoma. It has been suggested that such cytotoxic activities are due not only to killing activities derived from cytotoxic T-cells in vitro but also to tumor rejection in vivo (5).

These facts suggest that the HLA system or the human major histocompatibility complex may play a some role in the survival of cancer patients. Besides, there have been some reports that certain HLA types are associated with the prognosis of malignant diseases, e.g., HLA-A2 antigen for Hodgkin's disease (6), HLA-A9 antigen for acute lymphoblastic leukemia (7), and HLA-Aw19 and/or HLA-B5 antigens for lung cancer (8).

In our previous study, there were 17 postoperative stage III gastric cancer patients with chemotherapy or chemotherapy plus immunotherapy, who had survived for more than 5 years after gastrectomy. We speculate that the frequency of some HLA antigen of those survivors, especially those who received chemotherapy plus immunotherapy, is significantly different compared with those of the healthy controls and other gastric cancer patients.

This paper attempts to correlate the phenotype frequencies of HLA-A, -B, and -DR antigens with long-term survival of stage III gastric cancer.

**MATERIALS AND METHODS**

Seventeen patients with stage III gastric cancer who survived for more than 5 years after gastrectomy received the HLA typing test. Twelve of them received postoperative nonspecific immunotherapy plus chemotherapy, and the remaining patients received postoperative chemotherapy alone. The immunotherapy consisted of BCG-CWS injection, which was begun within the first postoperative week and given weekly during the first 2 months; subsequently each patient received monthly injection for as long a term as possible. The chemotherapy was initiated the first week after operation and the dosage schedule was 10 weekly injections of a combination of 2 mg mitomycin C, 250 mg 5-fluouracil, and 20 mg 1-β-d-arabinofuranosylcytosine, which is called MFC therapy in Japan. It was subsequently accompanied by daily administration of 600–800 mg Tegafur p.o. for as long as the patients survived.

As a control of those long-term survivors, 36 gastric cancer patients in all stages also received HLA typing test. Fourteen of them were diagnosed as having stage III gastric cancer.

HLA typing was performed by means of a standard microcytotoxicity technique (9, 10). Enriched B-lymphocytes were separated on a nylon wool column to determine DR antigens. The serotyping trays used for these tests were provided by Terasaki HLA-AB Oriental tray and DR tray (One Lambda, Inc., Los Angeles, CA) in addition to trays of the specific serum of Japanese furnished by National Sakura Hospital. As a control population, 139 healthy Japanese were typed for the A and B loci, and 64 others were typed for the DR locus. Eight antigens of the $A$ locus, 21 of the $B$ locus, and 10 antigens of the DR locus were tested on the lymphocytes. In HLA-DR antigens, lymphocytes with Cy6 antigen, associated HLA-DRw6 antigen, had MT2 but did not have DR3, DR5, DRw6, and DRw8 (11).

Statistical significance of phenotypic frequency of each antigen, using the $x^2$ test, was compared among the survivors, the healthy controls, and other gastric cancer patients.

**RESULTS**

Tables 1 to 3 show the HLA antigens and phenotypic frequencies found in the 17 survivors for HLA-A, -B, and -DR loci in comparison with the phenotypic frequencies of the healthy controls and other 36 gastric cancer patients including 14 stage III gastric cancer patients. Significant corresponding probabilities are summarized in Table 4.

The phenotypic frequency of HLA-A locus is shown in Table 1. For HLA-A2 antigen, patients with gastric cancer including those surviving had a low frequency compared with the healthy controls. However, no statistical difference was observed between the healthy control and the 17 survivors or other gastric cancer patients. In HLA-A9 antigen, those long-term survivors had a high frequency (94.1%) compared with healthy controls (64.0%) and other gastric cancer patients (63.9% for overall and 57.1% for stage III). However, no statistical difference, after correction of the $P$ value according to the number of HLA-A antigens tested, was observed between the healthy controls and those long-term survivors or other gastric cancer patients, as can be seen in Table 4.
The phenotypic frequency of HLA-B locus is shown in Table 2. Compared with healthy control and other gastric cancer patients, those survivors had a low frequency in HLA-Bw51 antigen, which was not statistically different. For HLA-Bw22 antigen, those survivors had a high frequency (17.6%) compared with healthy controls (2.9%) and other gastric cancer patients (2.8% for overall and 0% for stage III). However, it was also not statistically different after correction (Table 4).

Table 3 shows the phenotypic frequency of the HLA-DR locus. The phenotypic frequencies of the Japanese control population and 36 gastric cancer patients were 29.7 and 30.6% for HLA-DR4 antigen, respectively. On the other hand, that of 17 long-term survivors was 70.6% for HLA-DR4 antigen. The frequencies between the healthy control and those survivors with HLA-DR4 antigen were found to be significantly different ($P < 0.0025$). After correction of the $P$ value according to the number of antigens investigated, the association between the healthy control and the 17 survivors in HLA-DR4 antigen remained statistically significant, as shown in Table 4 (corrected $P < 0.025$).

**DISCUSSION**

Association of HLA type with the occurrence of a certain kind of disease has been recognized. For example, the frequency of normal controls with HLA-B27 antigen is low (9.4%), whereas patients with ankylosing spondylitis (90%) or Reiter's disease (79%) have a high frequency of HLA-B27 antigen. The relative risk of suffering from ankylosing spondylitis for individuals carrying HLA-B27 is 87.4, and from Reiter's disease is 37.0 (12). With technical advances of HLA-DR typing, associations of HLA-DR types with diseases has been received more attention. For example, HLA-DR3 antigen results in a relative risk of 15.4 for dermatitis herpetiformis, whereas HLA-DR2 antigen gives a relative risk of 15.9 for Goodpasture's syndrome (12).

In the patients with advanced stage III and IV gastric cancer, there were statistical differences between the control and the chemotherapy plus immunotherapy groups ($P < 0.05$) and between the chemotherapy and the chemotherapy plus immunotherapy groups ($P < 0.01$). In the patients with advanced stage III and IV gastric cancer, there were statistical differences between the control and immunotherapy groups. These results indicate that the beneficial effect of immunotherapy was particularly great in patients with advanced cancer. However, mechanisms of the effectiveness of nonspecific immunotherapy have not been fully understood.
that HLA-DR4 antigen in stage III gastric cancer patients may be related to longer survival.

The antigen frequency of HLA-DR4 based on our data of 64 normal Japanese subjects in this paper was 29.7%. On other hands, the DR4 antigen frequency was 41.4% based on data of 884 Japanese subjects in the report of Baur and Danilovs (16), and 34.8% based on data of 1998 Japanese subjects as reported at the Ninth Japan HLA Workshop, 1985 (17). DR4 antigen has not split yet, so those analyses were proper as well. This discrepancy may depend on the region difference and typing technique. If the figure of 41.4% is used as a control, the statistical difference will disappear.

Besides this, some reports have shown that certain HLA types are associated with the prognosis of malignant diseases, e.g., Hodgkin’s disease and HLA-B8 antigen (6), acute lymphoblastic leukemia and HLA-A9 antigen (7), and lung cancer and HLA-Aw19 and/or HLA-B5 antigens (8). In this study, the statistical difference in HLA-DR4 antigen has been observed between those survivors and the healthy controls, and even after correction of the P value according to the number of antigens tested, the statistical difference is still significant (P < 0.0025, corrected P < 0.025).

Continuation of this study will be directed to whether cytotoxic T-cells which have cytotoxic activities against syngeneic tumors by CML assay are highly induced in gastric cancer patients with HLA-DR4 antigen, as found in mice.

REFERENCES

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