HLA status and lymphocyte phenotypes in gastric cancer

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To evaluate the association between HLA status and cell-mediated immunity, we examined the lymphocyte phenotypes and the expression of 49 HLA antigens by lymphocytes in patients with gastric cancer.

Six hundred and six patients with histologically confirmed primary adenocarcinoma of the stomach underwent resection from March 1986 to October 1993. We classified these gastric cancer patients into four groups using a simple method of classifying patients according to HLA antigen status.

HLA type I patients had a low level of CD3 and CD4 cells and a high levels of CD16 cells, while HLA type III and IV patients had a high level of CD4 cells and a low level of CD16 cells. HLA type I and III patients, respectively, showed a decreased of CD57 and CD4 cells according to stage. HLA type II and IV patients did not show phenotypic changes of lymphocytes according to stage. These findings show that patients with different HLA types have a differences in immune status. A prospective randomized study using this new classification is now ongoing to confirm its clinical value in gastric cancer patients.

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The human major histocompatibility complex (MHC), also referred to as the human leucocyte antigen (HLA) complex, is located on a small part of chromosome 6. It is by far the most variable part of the genome and initiates immune responses¹⁾. Human leucocyte antigens are a group of cell membrane proteins encoded by the HLA complex with a high level of genetic polymorphism that regulate the immune response by self/not-self discrimination. MHC class I antigens are widely distributed on the membranes of almost every cell type, except for erythrocytes and spermatozoa. Unlike class I antigens, class II antigens are only expressed by certain kinds of cells. In humans, they are specifically expressed by macrophages (antigen-presenting cells), B cells, Langerhans cells, dendritic cells, activated T cells, and other cells of the immune system.

We have previously reported that expression of HLA A2 and B52 or DR4 antigens was respectively associated with a decreased and increased risk of lymph node metastasis in patients with gastric cancer^{2,3)}. We have also reported that Japanese gastric cancer patients could be divided into four types (I-IV) by classification according to HLA antigen status for easy analysis of HLA data in the clinical setting⁴⁾.

In the present study, we examined the lymphocyte phenotypes and HLA antigens expression by lymphocytes to evaluate the association between HLA type classified by information on only 21 HLA antigens, and cell-

mediated immunity in patients with gastric cancer.

Materials and methods

Six hundred and six patients with histologically confirmed primary adenocarcinoma of the stomach underwent resection from March 1986 to October 1993. Their ages ranged from 21 to 93 years (median : 60 years), and 70.0% were men. The patients were staged according to the 1987 Unio Internationalis Contra Cancrum (UICC) pTNM staging system⁵⁾.

Heparinized peripheral blood samples were obtained from the patients and were centrifuged for 5 min at 2000 RPM. After collecting the buffy coat, B lymphocytes were separated from T lymphocytes using nylon wool adherence and HLA class I and II Dynabeads (Dynal AS, Oslo, Norway). Peripheral blood lymphocytes were separated by layering onto Lymphoprep (Nycomed, Oslo, Norway) and centrifugation. For class I typing, cryopreserved peripheral blood lymphocytes were subjected to a complement-dependent microcytotoxicity assay using antisera to HLA-A, -B, and -C locus antigens. Class II typing was done on cells enriched for class II-positive lymphocytes using magnetic beads coated with a class II monoclonal antibody (Dynal, Oslo, Norway). These cells were subjected to a complement-dependent microcytotoxicity assay using antisera to HLA-DR and DQ antigens. Lymphocytes were typed for the following 49 antigens : A loci-1, 2, 3, 11, 24, 26, 31, 33 ; B loci-7, 13, 17, 27, 35, 37, 39, 44, 46, 48, 51, 52, 54, 55, 56, 59, 60, 61, 62, 67 ; C loci-w1, w3, w4, w6, w7 ; DR loci-1, 2, 3, 4, 5, 6, 7, 8, 9,

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Table 1 Results of statistical comparison among HLA types according to stage

1. stage 1A

	cd16%	cd3%	cd4%	CD4/8	cd57%	cd8%
Chi-Square	10.461	9.029	10.541	3.332	3.555	2.693
df	3	3	3	3	3	3
Asymp. Sig.	.015	.029	.014	.343	.314	.441

2. stage 1B

	cd16%	cd3%	cd4%	CD4/8	cd57%	cd8%
Chi-Square	.928	1.397	2.704	3.140	.362	3.796
df	3	3	3	3	3	3
Asymp. Sig.	.819	.706	.440	.371	.948	.284

3. stage 2

	cd16%	cd3%	cd4%	CD4/8	cd57%	cd8%
Chi-Square	3.521	2.927	.190	.911	2.417	.610
df	3	3	3	3	3	3
Asymp. Sig.	.318	.403	.979	.823	.490	.894

4. stage 3A, 3B

	cd16%	cd3%	cd4%	CD4/8	cd57%	cd8%
Chi-Square	4.326	7.428	1.837	.693	1.395	.108
df	3	3	3	3	3	3
Asymp. Sig.	.228	.059	.607	.875	.707	.991

5. stage 4

	cd16%	cd3%	cd4%	CD4/8	cd57%	cd8%
Chi-Square	2.511	.952	5.621	3.971	1.442	2.305
df	3	3	3	3	3	3
Asymp. Sig.	.473	.813	.132	.265	.696	.512

10, 12, 13; DQ loci-1, 2, 3, 4 All HLA data were obtained preoperatively by a single reference laboratory. The phenotypes of the lymphocytes were also examined by flow cytometry using an Ortho Cytron (Ortho Diagnostic Systems K. K., Tokyo, Japan) and the following antibodies: CD3, CD4, CD8, CD16 and CD57 (Ortho Diagnostic System K. K., Tokyo, Japan).

We classified the gastric cancer patients into four groups using a simple method of based on HLA antigen status. Type II patients had 2 or more HLA antigens from among A3, A33, B12, B13 and DR6, type III patients had 2 or more HLA antigens from among B46, B54, B55, B56, B59, Cw1, DR4 and DQ4, and type IV patients had 3 or more HLA antigens from among B7, B16, Cw7 and DR1. All other patients were type I. Type I is the commonest type in Japan and does not show any special characteristics.

Statistical analyses were carried out using SPSS 7.0J (SPSS Inc., Chicago, USA.). The nonparametric Kuskal-Wallis and Mann-Whitney U tests were used to determine the significance of differences and a significant difference was defined by $p < 0.05$.

Fig. 1 CD3 cells in stage 1A according to HLA type
HLA I vs III; $p=0.47$
HLA I vs IV; $p=0.28$
HLA II vs IV; $p=0.26$

Fig. 2 CD4 cells in stage 1A according to HLA type
HLA I vs III; $p=0.31$
HLA I vs IV; $p=0.22$
HLA II vs IV; $p=0.01$

Fig. 3 CD16 cells in stage 1A according to HLA type
HLA I vs IV; $p=0.02$

Results

By nonparametric analysis, the percentages of CD3, CD4, and CD16 cells showed significant differences among the HLA types in patients with stage 1A gastric cancer, but not in the other stages (Table 1). The percentages of CD8 and CD57 cells and the ratio CD4/8 did not show any significant differences in the other stages. Among stage 1A patients, HLA type III and IV patients showed higher levels of CD3 and CD4 cells than those with another HLA types (Fig. 1, 2), while HLA type I patients showed the highest level of CD16 cells (Fig. 3).

The percentage of CD57 and CD4 cells showed a significant difference among tumor stages in HLA type I and III patients, respectively (Table 2). Among HLA type

Table 2 Results of statistical comparison among stages according HLA types

1. HLA type I

	cd16%	cd3%	cd4%	CD4/8	cd57%	cd8%
Chi-Square	4.253	4.876	3.672	2.888	10.409	2.854
df	4	4	4	4	4	4
Asymp. Sig.	.373	.300	.452	.577	.034	.583

2. HLA type II

	cd16%	cd3%	cd4%	CD4/8	cd57%	cd8%
Chi-Square	3.932	2.739	3.822	2.924	7.525	1.707
df	4	4	4	4	4	4
Asymp. Sig.	.415	.602	.431	.571	.111	.790

3. HLA type III

	cd16%	cd3%	cd4%	CD4/8	cd57%	cd8%
Chi-Square	7.000	5.827	10.252	5.120	7.283	2.616
df	4	4	4	4	4	4
Asymp. Sig.	.136	.212	.036	.275	.122	.624

4. HLA type IV

	cd16%	cd3%	cd4%	CD4/8	cd57%	cd8%
Chi-Square	8.512	2.733	4.755	2.317	8.814	1.661
df	4	4	4	4	4	4
Asymp. Sig.	.075	.604	.313	.678	.066	.798

I patients, those with stage 1A disease had fewer CD57 cells than those other stages (Fig. 4). Among HLA type III patients, those with stage 1A disease showed a higher levels CD4 cells than those in other stages (Fig. 5).

Discussion

It has been reported that the CD4 coreceptor binds to class II antigens and the CD8 coreceptor binds to class I antigens. It has also been proposed that different HLA class I or class II molecules bind to distinct peptides. Moreover, the CD4-positive killer cell coreceptor also binds to MHC class II molecules^{6,7)}. Therefore, the oncologic significance of HLA antigens is an ongoing matter of debate. Each individual has unique and personal HLA antigens, so we selected HLA antigen status as a parameter of identification.

A recent study demonstrated that MHC class I-positive tumors stimulated autologous lymphocytes more frequently than did class I-negative tumors⁸⁾. In addition, the HLA B locus is adjacent to that for tumor necrosis factor^{9,10)}. These observations suggest that there may be an association between HLA phenotype and the effectiveness of anticancer therapy, especially immunotherapy.

The clinical implications of our HLA types may be as follows. The present study showed that HLA type I patients had a low level of CD3 and CD4 cells with a high

Fig. 4 CD57 cells in HLA type I patients according to stage
stage 1A vs 4; $p=0.01$
stage 1A vs 3A, 3B; $p=0.02$

Fig. 5 CD4 cells in HLA type III patients according to stage
stage 1A vs 4; $p=0.006$
stage 1A vs 1B; $p=0.02$

level of CD16 cells, while HLA type III and IV patients had a high level of CD4 and a low level of CD16 cells. In addition, HLA type I and III patients, respectively, showed a decrease of CD57 and CD4 cells according to stage, but type II and IV patients did not show any change of lymphocyte phenotype according to stages. Thus, the different HLA types showed a different immune status. We have previously presented results suggesting that HLA status could predict the response to anticancer therapy, and have proposed that rational individualized therapy for cancer patients might be designed by evaluating HLA antigens¹¹⁾. Development of parameters that correlate with the response of cancer to treatment would allow more rational and suitable use of anticancer therapy. A prospective randomized study using this new classification is now ongoing to confirm its clinical value in gastric cancer patients.

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