SHORT COMMUNICATION

Correlation between clinical response to interleukin 2 and HLA phenotypes in patients with metastatic renal cell carcinoma

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Summary HLA phenotypes were characterized for 79 patients with metastatic renal cell carcinoma treated with interleukin 2 (IL-2). HLA-A32 was associated with a clinical response (P=0.025). The frequency of HLA-A3 and/or A32 was higher among responders than non-responders (P=0.008). Thus, these results suggest that, in vivo, IL-2 may enhance cellular-mediated immunity against a tumour antigen and that some MHC molecules are more efficient than others for endogenous tumour antigen presentation.

Keywords: HLA; renal cell carcinoma; interleukin 2; immunotherapy

Recombinant Interleukin 2 (IL-2), alone or in combination with other agents, has been shown to induce tumour regression in 20–30% of patients with advanced melanoma or renal cell carcinoma (RCC) (Rosenberg et al, 1987; Négrier et al, 1989). Because of the severe side-effects observed during systemic administration of IL-2, it is important to identify the parameters that would predict clinical response. We have previously demonstrated that patients with metastatic RCC, for which high pretreatment levels of IL-6 were detected in the serum, had a very poor prognosis and did not respond to IL-2 treatment (Blay et al, 1992). The HLA phenotype represents another candidate for such a correlation. Major histocompatibility complex (MHC) products are important factors of the cellular arm of the immune response. Cytotoxic and helper T cells recognize processed antigenic peptides presented by MHC class I or II molecules respectively. The polymorphism of MHC proteins affects the ability of different alleles to bind with specific antigens; it is therefore likely that various HLA haplotypes differ in their ability to present tumour-specific endogenous antigens. Lilly et al, (1964) reported an association between histocompatibility antigens and susceptibility to virally induced leukaemia in mice. Further studies demonstrated that a relationship between susceptibility (Falk et al, 1971; Kantor et al, 1983) or resistance (Dellon et al, 1975; Oliver et al, 1977) to malignancy and HLA phenotype could be highlighted in human spontaneous tumours also, thus supporting the concept of immune surveillance in cancer patients. Further studies were based on these data to evaluate the association between HLA phenotypes and the likelihood of response to treatment, particularly in melanoma (Mitchell et al, 1992; Scheibenbogen et al, 1994; Marincola et al, 1995; Rubin et al, 1995). In the present study, we report a correlation between HLA distribution in RCC cancer patients and the predictability of response to IL-2 based therapy.

PATIENTS AND METHODS

Study population

The present study involved 79 metastatic RCC patients of European ancestry treated by immunotherapy with IL-2 after written informed consent. All patients were evaluable for response to IL-2. Characteristics of the patients, therapeutic regimens (West et al, 1987; Négrier et al, 1989; Atzpodien et al, 1990; Blay et al, 1992; Merrouche et al, 1995) and response to therapy are shown in Table 1.

HLA phenotyping

HLA phenotyping was performed on peripheral blood mononuclear cells using the standard microlymphocytotoxicity assay.

Statistical analysis

The frequencies of each single HLA antigen in the 79 patients were compared with those of a group of 124 normal volunteer blood donors of Caucasian origin for HLA class I phenotypes and a group of 192 donors for HLA class II phenotypes; all were typed by the same laboratory. The distribution of HLA phenotypes was then compared between responder and non-responder patients. Statistical analyses were performed using the Yates corrected chi-square test and the Fisher’s exact test (two-tailed). Each P-value herein reported should be multiplied by the number of antigens studied (i.e. 80) to correct for the selection of an antigen frequency occurring by chance alone. However, the P-values were reported uncorrected to facilitate comparison of these values with other investigations and because such a correction gives too conservative an estimate (Miller, 1981).
Table 1 Characteristics of the patients

| Characteristics | Male | (77%) | Female | (23%) |
|-----------------|------|-------|--------|-------|
| Age             | 61   | 18    |        |       |
| Median (year)   | 55   | 24–78 |        |       |
| Range (year)    | 0    | 41    | 1      | 27    |
| WHO performance status | 2 | 10 | 3 | 1 |
| CR, complete | 8     | 129   | 9      | 18    |
| Age             | 0     | 1     | >1     | 17    |
| Therapy regiment | 1 | 29 | 2 | 2 |
| IL-2 (i.v.)     | 16   | 34    | 10     | 52    |
| IL-2 (i.v.) + IFNα | 17 | 22 | 28 | 8 |
| IL-2 (i.v.) + LAK cells | 8 | 2 | 1 | 1 |
| Response to therapy | PR or CR | 28 | PD | 37 |
| CR, complete response | 16 | 23% | 24 | 30% |
| partial response | 18 | 23% | 37 | 47% |
| stable disease | 16 | 23% | 37 | 47% |
| progressive disease | 24 | 30% | 37 | 47% |

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

RESULTS

The comparison of the distribution of HLA phenotypes in the RCC population and in the control population of the same European Caucasian origin shows a significant difference for only one single-locus antigen, B51 (24% vs expected 12%, P = 0.027) (Table 2). The association of HLA phenotypes with clinical response to IL-2 has been investigated within the RCC population and is presented in Table 2. No statistical association was noted in this study between MHC class II phenotype and response.

HLA allele A32 was significantly correlated to response; 5 of the 18 (28%) responder patients were positive for HLA-A32 compared with 4 of 61 (7%) non-responder patients (P = 0.025). Furthermore, 8 of the 18 (44%) responder patients were positive for HLA-A3 compared with 14 of the 61 (23%) non-responder patients, a result which did not reach significance in this series (P = 0.075). A total of 11 of the 18 (61%) responder patients were A3 and/or A32 compared with 17 of the 61 (28%) non-responder patients (P = 0.008).

When patients were divided into either responders or stable and progressive-disease patients, an intermediate frequency in the expression of HLA-A3 and/or A32 was observed in stable-disease patients compared with responders and progressors (P = 0.027) (Table 3). Finally, the analysis of the overall 2-year survival in the responder and non-responder populations, comparing HLA-A3- and/or A32-positive vs negative patients, did not show a significant difference, but the number of patients was small (data not shown). A trend was observed within the responder population with a 2-year survival frequency of 61% for the HLA-A3- and/or A32-positive responder patients compared with a frequency of 28.5% in the HLA-A3- and/or A32-negative responding population.

Table 2 HLA phenotype frequencies in renal cell cancer patients, responder patients, non-responder patients and control population

| Allele | All patients n (%) | Control patients n (%) | Responders n (%) | Non-responders n (%) |
|--------|--------------------|-------------------------|------------------|----------------------|
| A1     | 18 22.8            | 33 26.6                 | 4 22.2           | 14 23.0              |
| A2     | 37 46.8            | 54 33.5                 | 9 26.4           | 28 32.0              |
| A3     | 22 27.8            | 31 25.0                 | 8 44.4           | 14 23.0              |
| A9     | 18 22.8            | 30 24.2                 | 5 27.8           | 13 21.3              |
| A10    | 6 7.6              | 12 9.7                  | 1 5.6            | 5 8.2                |
| A11    | 10 12.6            | 13 10.5                 | 3 16.7           | 7 11.5               |
| A23    | 4 5.1              | 4 3.2                   | 2 11.1           | 2 3.3                |
| A24    | 13 16.5            | 26 21.0                 | 3 16.7           | 10 16.4              |
| A25    | 1 1.3              | 4 3.2                   | 1 5.6            | 0 0.0                |
| A26    | 5 6.3              | 8 6.5                   | 0 0.0            | 5 8.2                |
| A28    | 9 11.4             | 11 8.8                  | 1 5.6            | 8 10.1               |
| A29    | 7 8.9              | 16 12.9                 | 1 5.6            | 6 9.8                |
| A30    | 2 2.5              | 7 5.6                   | 0 0.0            | 2 3.3                |
| A31    | 6 7.6              | 13 10.5                 | 1 5.6            | 5 8.2                |
| A32    | 9 11.4             | 12 9.7                  | 5 27.8           | 4 6.6                |
| A33    | 3 3.8              | 4 3.2                   | 0 0.0            | 3 4.9                |
| B5     | 20 25.3            | 19 15.3                 | 4 22.2           | 16 26.2              |
| B7     | 13 16.5            | 14 11.3                 | 4 22.2           | 9 14.8               |
| B8     | 10 12.7            | 19 15.3                 | 4 22.2           | 6 9.8                |
| B12    | 18 22.8            | 30 26.0                 | 3 16.7           | 15 24.6              |
| B13    | 2 2.5              | 3 2.4                   | 1 5.6            | 1 1.6                |
| B14    | 11 13.9            | 8 6.5                   | 3 15.6           | 8 13.1               |
| B15    | 9 11.4             | 16 12.9                 | 3 16.7           | 6 9.8                |
| B16    | 9 11.4             | 11 8.8                  | 0 0.0            | 9 14.8               |
| B17    | 4 5.1              | 10 8.1                  | 0 0.0            | 4 6.6                |
| B18    | 11 13.9            | 14 11.3                 | 4 22.2           | 7 11.5               |
| B19    | 5 6.3              | 5 4.0                   | 0 0.0            | 5 8.2                |
| B20    | 1 1.3              | 3 3.2                   | 1 5.6            | 0 0.0                |
| B42    | 0 0.0              | 1 0.8                   | 0 0.0            | 0 0.0                |
| B44    | 18 22.8            | 34 27.4                 | 3 16.7           | 15 24.6              |
| B45    | 0 0.0              | 4 3.2                   | 0 0.0            | 0 0.0                |
| B47    | 2 2.5              | 3 2.4                   | 0 0.0            | 2 3.3                |
| B49    | 3 3.8              | 9 7.6                   | 1 5.6            | 2 3.3                |
| B50    | 1 1.3              | 3 2.4                   | 0 0.0            | 1 1.6                |
| B51    | 19 24.1*           | 15 12.1*                | 4 22.2           | 15 24.6              |
| B52    | 0 0.0              | 4 3.2                   | 0 0.0            | 0 0.0                |
| B53    | 0 0.0              | 2 1.6                   | 0 0.0            | 0 0.0                |
| B54    | 4 5.1              | 4 3.2                   | 0 0.0            | 4 6.6                |
| B56    | 3 3.8              | 1 0.8                   | 2 11.1           | 1 1.6                |
| B57    | 4 5.1              | 7 5.6                   | 0 0.0            | 4 6.6                |
| B58    | 0 0.0              | 3 2.4                   | 0 0.0            | 0 0.0                |
| B60    | 8 10.1             | 11 8.8                  | 2 11.1           | 6 9.8                |
| B61    | 0 0.0              | 5 6.6                   | 0 0.0            | 3 4.9                |
| B62    | 9 11.4             | 13 10.5                 | 3 16.6           | 6 9.8                |
| B63    | 0 0.0              | 2 1.6                   | 0 0.0            | 0 0.0                |

The significance of the comparison was determined by the χ² test.

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

*P<0.027. **P<0.025.
Table 3  Association between clinical response and expression of HLA A3 and/or A32

| Number of patients (%) | CR+PR | SD | PD |
|------------------------|-------|----|----|
| A3- and/or A32-        | 11 (61%) | 8 (33%)  | 9 (24%) |
| A3 and A32             | 7 (39%)  | 16 (67%)  | 28 (76%) |
| Total                  | 18      | 24         | 37         |

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.  \( \chi^2 \)-test.

DISCUSSION

In the present study, the HLA profile of a group of 79 RCC patients was compared with a control population of Caucasian origin. Significant variations between the frequencies of HLA phenotypes in 35 patients with RCC and a control group of normal volunteer blood donors have already been reported. However, the elevated frequencies of HLA.Bw44 and HLA.DR8 observed in the group of RCC patients were respectively associated with familial RCC and the German or Scandinavian origin of the patients, a population group reported to have an elevated risk of RCC (Kantor et al, 1983). In contrast, in our series of patients, the higher frequency of B51 phenotype was not attributable to any peculiar clinical or ethnic characteristics.

Metastatic RCC, like melanoma, belongs to the small group of human tumours in which partial (or complete) remission has been observed in some patients after treatment with various forms of IL-2-based immunotherapy. In contrast to melanoma, cytotoxic T lymphocytes (CTL) showing MHC-restricted lysis of RCC have not been easily found among tumour-infiltrating lymphocytes (TIL). Nevertheless, some RCC have been shown to express antigenic determinants that could be specifically recognized by HLA.A2-restricted CTL (Schendel et al, 1993; Bernhard et al, 1994).

In this study, HLA.A32 is the only restriction element that significantly correlates with clinical response to IL-2; however, differences in the expression of HLA.A3 between responders and non-responders have also been noted. The association between some MHC phenotypes and response to therapy strongly suggests that in vivo, IL-2 may enhance cellular-mediated immunity directed against a tumour antigen and that some MHC determinants may be more efficient than others for endogenous tumour antigen presentation.

In this series, although the number of patients is too small to conclude, the HLA phenotype did not influence the overall survival of the responding population. Furthermore, when considering the HLA.A32 determinant individually, among the nine patients who were A32-positive (11.4%), four did not respond to IL-2 therapy. For HLA.A3 allele, the proportion of non-responding patients (14/22) is even greater. Thus, this parameter alone is not sufficient to delineate the subgroup of patients that would benefit from IL-2 therapy. These data suggest that, in RCC, mechanisms other than the processing of tumour antigen can lead to immunosuppression and tumour progression. Although these mechanisms have not been completely elucidated, structural and functional alterations in lymphocytes infiltrating RCC tumours have been recently described. Other mechanisms, such as the production of immunosuppressive cytokines (Wang et al, 1995; Ménétrier-Caux et al submitted for publication), alterations in T-cell signal transduction (Finke et al, 1993) or an inefficient co-stimulation by accessory molecules (Bain et al, 1996), can also be put forward.

Prospective analyses of a larger series of patients are needed in order to validate these results.

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