Antigens recognized by T-lymphocytes on human tumours

P. G. Coulie*, B. J. Van den Eynde, P. van der Bruggen, A. Van Pel and T. Boon

Cellular Genetics Unit, Université Catholique de Louvain, Brussels, Belgium and Ludwig Institute for Cancer Research, B1200 Brussels, Belgium

Tumour-specific cytolytic T-lymphocytes (CTLs) have been obtained in several human tumour types, mainly melanoma. By using a genetic approach based on the transfection of genomic or cDNA libraries, the genes encoding a number of human tumour antigens have been cloned. This has led to the identification of antigenic peptides presented by tumour cells on various HLA class-I molecules. On the basis of their pattern of expression, these antigens can be classified into three groups. Antigens in the first group are encoded by genes that are expressed in many tumour cells but are silent in normal adult tissues except testis. The second group consists of differentiation antigens. Antigens of the third group result from mutations occurring in genes that are expressed in most normal tissues.

It is worth noting that other types of tumour antigen that are recognized by CTLs have been characterized: polymorphic epithelial mucins, viral antigens such as those encoded by human papilloma viruses, and antigens encoded by genes that are overexpressed in tumours such as HER-2/ neu. This brief review does not cover these last three categories of tumour antigens.

Tumour-specific shared antigens

Three families of genes that appear to code for highly specific tumour antigens have been identified so far, namely the MAGE, BAGE and GAGE genes [1–4]. These genes are frequently expressed in a wide range of tumour types such as melanoma, lung carcinoma, sarcoma and bladder carcinoma but very rarely in other tumour types such as brain tumours, renal carcinoma and leukaemia (Table 1) [2,5–7]. The only normal tissues in which expression of these genes has been observed are testis and placenta [2]. Starting from CTL clones obtained by stimulating lymphocytes with an autologous melanoma cell line, eight antigens encoded by MAGE-1, MAGE-3, BAGE and GAGE have been identified [3,4,8–12]. For these eight antigens,

Abbreviation used: CTL, cytolytic T-lymphocyte.
*To whom correspondence should be addressed.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>MAGE-1</th>
<th>MAGE-3</th>
<th>BAGE</th>
<th>GAGE-1, 2</th>
<th>RAGE-1</th>
</tr>
</thead>
</table>
| Melanoma
  Primary lesions            | 16     | 36     | 8    | 13        | 2      |
  Metastases                  | 48     | 76     | 26   | 28        | 5      |
| Non-small cell lung carcinoma| 49     | 47     | 4    | 19        | 0      |
| Head and neck tumours       | 28     | 49     | 8    | 19        | 2      |
| Bladder carcinoma           | 22     | 36     | 15   | 12        | 5      |
| Sarcoma                     | 14     | 24     | 6    | 25        | 14     |
| Mammary carcinoma           | 18     | 11     | 10   | 9         | 1      |
| Prostatic carcinoma         | 15     | 15     | 0    | 10        | 0      |
| Colorectal carcinoma        | 2      | 17     | 0    | 0         | 0      |
| Renal carcinoma             | 0      | 0      | 0    | 0         | 2      |
| Leukaemia and lymphoma      | 0      | 0      | 0    | 1         | 0      |
| Testicular seminoma         | 4 of 6 | 3 of 6 | 1 of 6 | 5 of 6 | 0 of 3 |

Expression of genes MAGE-1 and -3, BAGE, GAGE and RAGE in tumour samples

Expression was measured by reverse transcription and PCR on total RNA using primers specific for each gene. Results are expressed as percentages except for testicular seminoma.

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both the presenting HLA molecule and the antigenic peptide have been completely defined (Table 2).

The MAGE gene family contains at least 12 genes which share 64–85% identity [2]. They are located on the long arm of chromosome X, in the Xq28 region. The function of the MAGE genes is still unknown. However, observations on the main hydrophobic regions throughout the MAGE family suggest that all corresponding proteins may have closely related functions. In contrast, the promoters and first few exons of the 12 MAGE genes show great variability. A similar function could therefore be regulated under different transcriptional controls, thus occurring at specific times and locations.

More than 60% of Caucasian patients with melanoma bear one of the presently defined antigens encoded by MAGE, BAGE and GAGE. For other cancers such as lung cancer, head and neck cancer and bladder cancer, the frequencies range from 40 to 28%. It appears increasingly unlikely that immunization of patients against one of these antigens will cause harmful immunological side effects due to the expression of the relevant gene in the testis. This expression appears to occur in germline cells, more precisely spermatocytes and spermatogonia [15]. A similar observation has been made with the mouse equivalent of a MAGE gene by in situ hybridization [16]. Because these germline cells do not express MHC class-I molecules, gene expression should not result in antigen expression [17]. These conclusions are further strengthened by immunization studies carried out with mouse tumour antigen P815A, which is encoded by a gene that is also expressed only in testis. After immunization with P815 tumour cells, which carry this antigen, male mice produced a strong CTL response. No inflammation of the testis was observed in the following months and the fertility of these mice was normal [18].

A new mode of origin for antigens that are also tumour-specific shared antigens has been recently described [14]. Here, a gene that is ubiquitously expressed, namely one encoding N-acetylglucosaminyltransferase V, contains an intron that itself appears to carry near its end a promoter that is activated only in melanoma cells. This atypical activation occurs in more than 50% of melanomas. It produces a message containing a new open reading frame, which codes for the antigenic peptide in its intronic part. This peptide is presented by HLA-A2 to melanoma-specific CTLs.

Recently, a new antigen has been identified that is recognized by CTLs on a kidney tumour. This antigen was found to be encoded by a previously unknown gene that we called RAGE [13]. This gene is silent in normal tissues except retina, and is expressed in a small proportion of tumours, mainly sarcomas, bladder tumours and melanomas (Table 1). Since most retinal cells do not express MHC class-I molecules, this antigen is probably tumour-specific, although the formal proof of this will require the identification of the retinal cell type that expresses RAGE. The antigenic peptide recognized by the CTLs has been identified. It is presented by HLA-B7 (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normal expression</th>
<th>MHC</th>
<th>Peptide</th>
<th>Position</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAGE-1</td>
<td>Testis</td>
<td>HLA-A1</td>
<td>EADPTGHSY</td>
<td>161–169</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLA-Cw16</td>
<td>SAYGEPRKL</td>
<td>230–238</td>
<td>9</td>
</tr>
<tr>
<td>MAGE-3</td>
<td>Testis</td>
<td>HLA-A1</td>
<td>EVDPIGHLY</td>
<td>168–176</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLA-A2</td>
<td>FLWGPRLALV</td>
<td>271–279</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLA-B44</td>
<td>MEVDPIGHLY</td>
<td>167–176</td>
<td>11</td>
</tr>
<tr>
<td>BAGE</td>
<td>Testis</td>
<td>HLA-Cw16</td>
<td>AARAVFLAL</td>
<td>2–10</td>
<td>3</td>
</tr>
<tr>
<td>GAGE-1/2</td>
<td>Testis</td>
<td>HLA-Cw6</td>
<td>YRPRPRRY</td>
<td>9–16</td>
<td>4</td>
</tr>
<tr>
<td>RAGE-1</td>
<td>Retina</td>
<td>HLA-B7</td>
<td>SPSSNIRNT</td>
<td>11–20</td>
<td>13</td>
</tr>
<tr>
<td>GnT4 (atypical transcript)</td>
<td>None</td>
<td>HLA-A2</td>
<td>VPDVIRNC</td>
<td>38–64</td>
<td>14</td>
</tr>
</tbody>
</table>
Differentiation antigens

The observation that several CTLs generated against autologous melanoma cells were recognizing differentiation antigens present also on normal melanocytes was unexpected. Four genes encoding melanoma differentiation antigens have been identified: tyrosinase, Melan-A/Mart-1, gpl00 and gp75 [19-24]. Most of the identified antigenic peptides are presented by HLA-A2, but other HLA–peptide combinations have been found [23-32]. One tyrosinase peptide is presented by HLA-DR4 to CD4 T-cells [28].

A tyrosinase peptide presented by HLA-A2 presents an interesting post-translational modification. An asparagine residue is transformed into an aspartic acid residue, presumably by the asparagine being glycosylated and subsequently deglycosylated by an enzyme that removed the amino group with the glycan [33].

There is concern for the potential side effects of active or passive immunization against melanoma-differentiation antigens, not so much for the skin, where vitiligo due to the destruction of melanocytes might occur, but for the retina where melanocytes are present in the choroid layer. However, vitiligo has been associated with good prognoses in melanoma and also with adoptive transfer of tumour-infiltrating lymphocytes, without noticeable eye lesions [29,31,34,35]. Carefully devised immunotherapy trials based on these antigens would therefore appear to be permissible.

Antigens encoded by mutated genes

Point mutations also generate antigens recognized on melanoma by autologous CTLs. As was seen with the mouse antigens induced by mutagens, the mutations are located in the region coding for the antigenic peptide, enabling it to bind to the MHC molecule or generating a new epitope. A very interesting example is the point mutation of cyclin-dependent kinase 4 [36], which prevents this protein from binding to p16, thereby increasing the probability of it binding to the cyclin molecule and phosphorylating the retinoblastoma gene product Rb, so that the E2F transcription factor is released and activates genes required for entry into the S phase of the cell cycle. This is clearly a mutation that is both antigenic and oncogenic. The oncogenic potential of this mutation is underscored by the observation that one of 28 other melanomas that were tested carried the same mutation. The amino acid change generated by this mutation enables the peptide to bind to the HLA-A2-presenting molecule.

Another interesting point mutation produces a new antigenic peptide which, remarkably, is partially encoded by the 5' end of an intron [37]. In this instance, the mutation generates a new epitope in a peptide that binds to HLA-B44. A recent report describes an antigenic peptide produced by a mutation in the β-catenin gene, which codes for a cell-surface adhesion molecule. This mutation creates an anchor residue enabling the peptide to bind to HLA-A24 [38].

Point mutations may also create tumour antigens by directly altering an HLA molecule: we recently observed that autologous CTLs directed against a human renal cell carcinoma recognized an HLA-A2 molecule that was altered as a result of a point mutation changing one amino acid in the α-2 helix [39].

The antigens generated by point mutations ought to be absolutely specific for the tumour cells, and the CTL precursors directed against these antigens should not have undergone any of the depletion or anergy that accompany natural tolerance. On the other hand, they are expected to be unique for an individual tumour or restricted to very few. This should make it difficult to develop cancer therapeutic vaccines based on these antigens. But one should not exclude the possibility that technological progress may one day make the identification of such antigens so easy that strictly individual immunogens will become a realistic possibility.

Towards vaccination?

Some of the tumour antigens that we have mentioned are in the early stages of clinical study. There is little doubt that the coming years will witness a large number of clinical trials involving peptides, proteins and recombinant defective viruses.

The knowledge of the molecular nature of these antigens first allows the selection of patients whose tumour actually expresses a given antigen. Eligible tumours should express the relevant gene along with the appropriate HLA class I specificity, and this can be tested readily by reverse transcription and PCR amplification on RNA extracted from a small tumour sample. The fraction of tumours expressing a given antigen can be calculated from the frequency of expression of the relevant gene in that tumour type and from the frequency of the given class I molecule in the population.
Secondly, the definition of the molecular nature of tumour antigens allows the rational design of highly specific vaccine preparations. These could consist of either engineered cells expressing the antigens or antigenic peptides mixed with appropriate adjuvants. The availability of the genes encoding the antigens also allows the preparation of recombinant proteins that can be combined with adjuvants, or the preparation of recombinant viral vectors that can be used for immunization.

It appears that responses have been obtained in some patients with melanoma immunized with a MAGE-3 peptide presented by HLA-A1 [40]. It is our hope that the careful study of the lymphocytes and tumour cells of these patients will produce a rich harvest of additional antigens and a better understanding of what constitutes an effective anti-tumour response.

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