

Review

TUMOUR-ASSOCIATED ANTIGENS IN MULTIPLE MYELOMA

Multiple myeloma (MM) is a fatal haematological malignancy characterized by an accumulation of monoclonal malignant plasma cells within bone marrow (Bataille & Harousseau, 1997). The most efficient treatment for patients under 65 years of age is intensive therapy (high-dose melphalan followed by autologous stem cell transplantation) (Attal *et al*, 1996), which improves the remission rate, event-free survival and overall survival, but does not lead to a cure (remissions are not maintained because of the persistence of a residual disease). Thus, there is an obvious need for new treatments to stabilize or even eradicate residual disease. Immunotherapy could be the appropriate means of controlling residual disease as well as providing an alternative treatment to conventional chemotherapy for patients over 65 years of age. To date, MM immunotherapy trials have only involved immunoglobulin idiotype (Id) vaccination, as no other tumour-associated antigen (TAA) has yet been identified (Stevenson & Anderson, 2000). However, vaccination with Id, a private TAA, cannot provide shared immunotherapy for MM patients. In this context, it is difficult to characterize Id-specific cytotoxic T lymphocytes (CTL) (restriction, peptides, etc.) in patients. Various studies have shown that idiotype-induced CTL can kill myeloma cells, but there is no direct evidence that these CTL recognize a peptide derived from idiotype (Lim & Bailey-Wood, 1999; Reichardt *et al*, 1999; Li *et al*, 2000; Titzer *et al*, 2000). More recently, Trojan *et al* (2000) reported that peptides derived from the Ig framework elicited a shared CTL recognition of CD40-activated B cells and malignant B cells from CLL (chronic lymphocytic leukaemia) but no data were provided for malignant plasma cells. In recent years, the expression of TAA coded by cancer germ line-specific or over-expressed genes has been detected in myeloma cells, which opens up a new and promising field for immunological trials in MM. The purpose of this review was to consider these newly identified shared TAA in MM.

Three approaches have been used to identify TAA: (i) isolation of tumour-specific CTL from patients and cloning of a gene encoding the peptide (Robbins & Kawakami, 1996; Boon *et al*, 1997; Rosenberg, 1999); (ii) so-called reverse immunology by designation of the peptide (occasionally elution peptide from tumour cells) and generation of CTL (Flad *et al*, 1998; Kessler *et al*, 2001; Pinilla *et al*, 2001); (iii) cloning of genes from tumour prokaryotically expressed

cDNA libraries using autologous antibodies of patients (serological analysis of recombinant cDNA expression libraries, SEREX) (Tureci *et al*, 1997).

The tumour-specific genes coding for peptides recognized by T cells (recently reviewed by Renkvist *et al*, 2001) include normal genes, widely or tissue-specific over-expressed genes (PRAME, tyrosinase), cancer germ line-specific genes (MAGE), mutated genes (ras) and aberrantly expressed sequences from normal genes (intronic, alternative ORF, fusion induced by chromosomal translocations such as BCR/ABL).

For MM, all TAA identified to date have been found by reverse transcription polymerase chain reaction screening of gene expression, although studies using CTL or SEREX approaches are continuing.

An attempt was made to classify TAA according to their potential interest for MM immunotherapy. An arbitrary score was determined as the sum of an expression score (/5) and a CTL recognition score (/5) (see the legend to Table I).

OVER-EXPRESSED GENES

MUC1 was the first TAA described in MM. Myeloma cells were shown to express the mucin MUC1 recognized by T cells in a human leucocyte antigen (HLA)-unrestricted, but also restricted, manner (Takahashi *et al*, 1994; Brossart *et al*, 2001). MUC1 is frequently expressed by bone marrow myeloma cells but often by a subset of cells (unpublished observations). Anti-MUC1 T lymphocytes can kill myeloma cells efficiently, but may also kill non-malignant cells such as epithelial cells (Takahashi *et al*, 1994) or normal plasma cells (unpublished observations) that express MUC1. MUC1 is a very good candidate for immunotherapy of myeloma (score 9/10, Table I). It is uncertain whether anti-MUC1 vaccination would be specific in MM, although preliminary vaccination trials with MUC1 peptides of patients bearing breast and ovarian cancers did not show any side-effects or autoimmune reactions (Brossart *et al*, 2000).

The PRAME gene encodes an antigen recognized on a human melanoma cell line by an autologous CTL clone in HLA-A24 restriction (Ikeda *et al*, 1997). This gene (located in chromosome 22) is expressed at a high level in tumours, such as melanomas, carcinomas and sarcomas, and at low levels in normal tissues. In haematological malignancies, PRAME is expressed in acute myeloblastic leukaemia carrying the t(8;21) translocation, which fuses the AML1 and ETO genes, and in MM (van Baren *et al*, 1998, 1999; Pellat-Deceunynck *et al*, 2000). In a recent study, Kessler *et al* (2001) showed that four PRAME epitopes are presented by HLA-A*0201 to specific CTL that lyse various tumour

Correspondence: Catherine Pellat-Deceunynck, Inserm u463, Equipe labellisée par la Ligue Contre le Cancer, Institut de Biologie, 9 Quai Moncousu, 44093 Nantes cedex 01, France. E-mail: cpellat@nantes.inserm.fr

Table I. Tumour-associated antigens in multiple myeloma.

	Expression in myeloma cells from			Specific CTL lysis of			Overall score/10
	Cell lines	Patients	Score/5	Myeloma cells	Tumour cells	Score/5	
Over-expressed genes							
MUC1	100% (n = 8) ^{a,b,c}	63% (n = 30) ^c	4	+ ^d	+	5	9*
hTERT	100% (n = 1) ^e	nd	/	+ ^e	+	5	/
Sp17	89% (n = 9) ^{f,g}	17% (n = 41) ^f	1	+ ^f	+	5	6*
Prame	80% (n = 16) ^h	43% (n = 61) ^{h,i}	3	nd	+	4	7*
Cancer germ line-specific genes							
MAGE-A1	100% (n = 16) ^h	37% (n = 61) ^{h,i}	2	+ ^h	+	5	7
MAGE-A2	nd	27% (n = 40) ⁱ	2	nd	+	4	6
MAGE-A3	81% (n = 16) ^h	24% (n = 61) ^{h,i}	2	+ ⁱ	+	5	7
MAGE-A4	nd	15% (n = 40) ⁱ	1	nd	+	4	5
MAGE-A6	nd	25% (n = 40) ⁱ	2	nd	+	4	6
MAGE-A10	nd	7% (n = 40) ⁱ	1	nd	+	4	5
MAGE-A12	nd	15% (n = 40) ⁱ	1	nd	+	4	5
BAGE	69% (n = 16) ^h	13% (n = 61) ^{h,i}	1	nd	+	4	5
GAGE-1,2	50% (n = 16) ^h	17% (n = 61) ^{h,i}	1	nd	+	4	5
GAGE-3,6	nd	40% (n = 40) ⁱ	3	nd	nd	/	/
LAGE-1	nd	47% (n = 40) ⁱ	3	nd	+	4	7
NY-ESO-1	nd	27% (n = 40) ⁱ	2	nd	+	4	6
Mutated genes							
Ras	50% (n = 16) ^j	53% (n = 43) ^j	3	nd	+	4	7
P53	50% (n = 10) ^k	16% (n = 82) ^{k,l}	1	nd	+	4	5
FGFR3	17% (n = 12) ^m	2% (n = 53) ⁿ	1	nd	nd	/	/

*risk of autoimmunity.

a, Takahashi *et al* (1994); b, Noto *et al* (1997); c, unpublished observations; d, Brossart *et al* (2001); e, Vonderheide *et al* (1999); f, Lim *et al* (2001); g, unpublished observations; h, Pellat-Deceunynck *et al* (2000); i, van Baren *et al* (1999); j, Bezieau *et al* (2001); k, Portier *et al* (1992); l, Neri *et al* (1993); m, Chesi *et al* (1997); n, Intini *et al* (2001).

nd, not done; /, no score. Expression score in patients with MM: 0 (0%), 1 (1–19%), 2 (20–39%), 3 (40–59%), 4 (60–79%), 5 (80–100%). CTL lysis score: 4 lysis of myeloma cells not reported but lysis of tumour cells, 5 lysis of myeloma cells reported.

cells expressing PRAME and HLA-A*0201. The authors identified PRAME as an attractive target for cancer immunotherapy despite the widespread (although weak) expression of the gene in normal tissues. PRAME could also be of major interest in MM as it is frequently expressed (score 7/10, Table I).

Telomerase, a ribonucleoprotein involved in the homeostasis of chromosome length, is widely expressed by cancer cells (also by bone marrow stem cells) and over-expressed as compared with normal cells. The catalytic subunit (hTERT) is a TAA recognized by CTL in the U266 myeloma cell line (Vonderheide *et al*, 1999). Moreover, hTERT-specific CTL from patients with cancer (including MM) can be generated *in vitro* (Vonderheide *et al*, 2001). Nevertheless, the over-expression of hTERT was not addressed in primary myeloma cells. Clinical trials are now needed to define whether hTERT is a suitable, safe TAA for immunotherapy of patients with cancer, including MM.

Sperm protein 17, a spermatozoa-restricted protein expressed by myeloma cells (Chiriva-Internati *et al*, 2001; Lacy & Sanderson, 2001; Lim *et al*, 2001), is immunogenic, because normal vasectomised men raise antibodies against it (Lea *et al*, 1997). Moreover, HLA-I-restricted Sp17-specific

CTL kill Sp17⁺ HLA-A1⁺ myeloma cells (Chiriva-Internati *et al*, 2001). Lacy & Sanderson (2001) found that Sp17 was expressed by both normal and malignant lymphoid cells, which suggests that Sp17 is not a true TAA. Nevertheless, as normal lymphoid cells show lower Sp17 expression than myeloma cells, Sp17 may constitute a suitable TAA for MM patients. Anyway, as for hTERT, it remains to be determined whether Sp17 would be safe for immunotherapy of patients with MM.

The use of differentiation TAA (such as melanA) to vaccinate patients with melanoma has shown that vitiligo is often associated with antitumour responses. This suggests that efficient immunological responses necessarily induce some autoimmunity. In other words, TAA susceptible of inducing autoimmunity are still interesting targets, provided that autoimmunity remains limited to some tissues or is controllable (adoptive transfer of TK⁺ T lymphocytes, etc.).

CANCER GERM LINE-SPECIFIC GENES

MAGE-A1, the first cloned human TAA recognized by T cells, was isolated using T cells derived from a melanoma

patient immunized by autologous cancer cells (van der Bruggen *et al.*, 1991). It was determined that MAGE-A1 belongs to a family of 12 genes (MAGEA1-A12) located in the q28 region of chromosome X (Gaugler *et al.*, 1994; Chomez *et al.*, 2001). Other members of the cancer germ line family are BAGE, GAGE, LAGE-1 and NY-ESO-1 (Van den Eynde *et al.*, 1995; Chen *et al.*, 1997; Lethe *et al.*, 1998; De Backer *et al.*, 1999). Chromosomal localization of these genes, when available, indicates that they are found in chromosome X (mainly Xq28). In adults, their expression is restricted to cancer cells and male germ cells (with some showing weak expression in placenta or ovary), although peptides are not presented in testis because of a lack of major histocompatibility complex molecules. Expression of cancer germ line-specific genes is controlled by DNA methylation of CpG islands of their promoter (De Smet *et al.*, 1999). Cancer germ line-specific genes are expressed in various tumour cells (melanoma, carcinoma), but not in haematological malignancies other than MM (van Baren *et al.*, 1999; Pellat-Deceunynck *et al.*, 2000). In MM, cancer germ line-specific genes are preferentially expressed in advanced disease (up to 50%) and in almost all cell lines. All the genes of the MAGE-A family screened were found to be expressed in myeloma cells, although with various incidences (Table I). As MAGE-A1- and MAGE-A3-specific CTL lyse MM cell lines, and as both proteins are expressed in primary myeloma cells, MAGE-A1 and MAGE-A3 would seem to be very good candidates for immunotherapy of patients with MM (score 7/10, Table I). However, other members such as NY-ESO-1 or LAGE-1 could also be of interest, although it remains to be determined whether specific CTL are able to recognize myeloma cells.

MUTATED GENES

Mutations in codons 12, 13 and 61 of N-ras and K-ras genes are frequent in MM (Portier *et al.*, 1992; Bezieau *et al.*, 2001; Kalakonda *et al.*, 2001). At codon 61, a basic amino acid such as leucine, arginine or lysine is substituted for glutamine (Bezieau *et al.*, 2001; Kalakonda *et al.*, 2001). Peptides derived from mutated ras genes bind to HLA class I and II molecules, and specific CTL have been induced *in vitro* and even isolated from a patient with carcinoma (Gedde-Dahl *et al.*, 1992; Juretic *et al.*, 1996). Mutated ras-based immunotherapy could be of interest in MM, although the mutations involve all three codons and there is not a single amino acid substitution for each codon (score 7/10, Table I). It remains to be determined which mutated peptides bind to the most frequent HLA alleles and whether specific CTL could be generated.

Mutations in the p53 gene were observed in less than 20% of patients studied (Portier *et al.*, 1992; Neri *et al.*, 1993). Mutations in the FGFR3 gene were observed in 17% of MM cell lines but in only one patient out of 53, i.e. 2% (Chesi *et al.*, 1997; Intini *et al.*, 2001). This patient had a (4;14) translocation which is found in about 20% of patients. These two incidences are too low to take p53 or

FGFR3 mutations into account for the immunotherapy of patients with MM.

IDENTIFICATION OF OTHER MYELOMA-SPECIFIC GENES

Over-expressed and tumour-specific genes

The cancer germ line-specific LAGE-1 gene was cloned by subtractive cDNA libraries between melanoma and normal skin. In the era of DNA arrays, other normal genes, either widely expressed, tumour-specific or lineage-specific, will be identified (De Vos *et al.*, 2001; Magrangeas *et al.*, 2001). Identification of the over-expressed genes involved in oncogenesis or tumour expansion of MM could be the basis for development of second-generation tumour antigens, as proposed by Schultze & Vonderheide (2001).

Over-expressed and chimaeric genes generated by recurrent chromosomal rearrangements

It has been clearly established that malignant plasma cells have chromosomal translocations involving switch regions of the IgH gene (Bergsagel & Kuehl, 2001). In contrast to other haematological malignancies such as Burkitt's lymphoma, MM is not characterized by a recurrent translocation involving a single dysregulated gene (Avet-Loiseau *et al.*, 1999). On the contrary, multiple partners have been found on different chromosomes: cyclin D1 on chromosome 11 (Chesi *et al.*, 1996), FGFR3, MMSET/WHSC1 on chromosome 4 (Chesi *et al.*, 1998a; Stec *et al.*, 1998), c-maf on chromosome 16 (Chesi *et al.*, 1998b), MUM1/IRF4 and cyclin D3 on chromosome 6 (Iida *et al.*, 1997; Shaughnessy *et al.*, 2001). Translocations that do not involve Ig loci, such as t(8;?) involving c-myc and l-myc genes were recently described (Shou *et al.*, 2000; Avet-Loiseau *et al.*, 2001). Although few data are available, translocations induce over-expression of genes (cyclin D1, FGFR3) in most cases. However, hybrid transcripts of IgH and MMSET/WHSC1 have also been reported. The detection of fusion protein was not considered, but it is possible that some translocations generate at least hybrid peptides that could be targeted as specific TAA, as recently described for BCR/ABL in chronic myeloid leukaemia (Clark *et al.*, 2001). As translocation of one gene is involved in less than 20% of patients and because there are no data available concerning their over-expression, we cannot conclude yet that one gene is or is not of particular interest for a shared immunotherapy of patients with MM. Nevertheless, genes involved in recurrent translocations such as cyclinD1 or FGFR3 could be of major interest for the subset of patients carrying the translocation t(11;14) or t(4;14).

Murine myeloma-specific antigen

In the 1980s, mouse models allowed the demonstration of CTL recognition of tumour cells, leading to the discovery of TAA in mouse and human. The lack of a specific murine model has long been a limiting factor in MM studies and a true equivalent (5T2) has only been described recently (Vanderkerken *et al.*, 1996; Croucher *et al.*, 2001). It would

be of interest to isolate 5T2-specific CTL and identify genes coding for these antigens.

Identification of genes encoding peptides recognized by myeloma-specific CTL

Van der Bruggen *et al* (1991) described the cloning of a gene-encoding peptide recognized by CTL on human melanoma. This approach is the only one to enable the identification of all types of TAA coded by cancer germ line-specific genes, over-expressed genes, mutated genes, alternatively expressed genes (cryptic ORF, intron expression), etc. This approach (now classic) has enabled the cloning of numerous genes in different cancers, but remains particularly difficult in MM because of the inaccessibility of biological material. Ordinarily, it is impossible for the great majority of primary myeloma cells (> 99.9%) to establish a cell line and the frequent collection of fresh bone marrow cells is unethical. However, our group was able to derive a CTL clone in a particular patient with solitary plasmacytoma that recurred in the skin (an antigenic site) and then in pleura. Pleural effusion enabled the establishment of a cell line and the patient was cured by pleura removal. Two years later, he was still in remission, and it was possible to derive CTL clones after co-culture of PBL with the tumour cell line (Pellat-Deceunynck *et al*, 1999). The HLA-A*0201-restricted clone proved of particular interest as it also recognized U266 (in HLA-A*0201 restriction), but not HLA-A02⁺ melanoma cell lines, which suggests that it recognizes a potentially myeloma-specific TAA (unpublished observations). With the development of dendritic cell engineering, it is now easier to derive tumour-specific CTL *in vitro*, as reported in recent studies in which dendritic cells pulsed with myeloma cells were used to generate myeloma-specific CTL (Yi *et al*, 2001; Dhodapkar *et al*, 2002). Myeloma-specific CTL will necessarily allow the identification of myeloma-expressed TAA.

CONCLUSION

To date, no ideal TAA exists for MM, although MUC1 seems to be a very good candidate. The only well-expressed, fully tumour-restricted TAA is monoclonal Id, although Id vaccinations have not shown significant clinical response. The cancer germ line-specific gene family constitutes another fully tumour-restricted TAA, but the expression of any given member concerns less than 50% of patients. The other TAA described are not fully restricted to tumour cells and could induce autoimmune disorders that have to be evaluated. To overcome the escape of variants, trials involving more than one TAA need to be designed, although Brossart *et al* (2000) reported that vaccinations of patients with MUC-1 or HER-2/Neu peptides (loaded on dendritic cells) elicited not only specific CTL responses but also anti-MAGE or anti-ACE CTL responses, indicating that an epitope spreading occurred *in vivo*. Different MAGE family proteins could be combined together or with over-expressed genes such as PRAME. Another approach already developed for other cancers would involve the vaccination of patients with killed autologous myeloma cells or well-defined allogeneic

myeloma cell lines expressing different TAA. MM patients show epidemiological, biological and clinical heterogeneity, and the genetic bases of this heterogeneity are beginning to be elucidated (Avet-Loiseau *et al*, 2002; Zhan *et al*, 2002). In this context, it seems difficult to designate a single TAA shared by all patients. In other words, it will be critical to establish the precise TAA expression profile in each subset of patients defined by biological and genetic parameters (microarray profile, recurrent translocations, etc.) in order to propose an immunological trial shared between patients within each subset. In any event, the immunotherapy of patients with MM, which has been limited by a lack of shared TAA, is about to enter a new era.

INSERM U463, CATHERINE PELLAT-DECEUNYNCK
Equipe labellisée par
la Ligue Contre
le Cancer, Institut
de Biologie, Nantes,
France. E-mail: cpellat@
nantes.inserm.fr

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