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Active immunotherapy of multiple myeloma

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ABSTRACT

Since myeloma cells express various potential target antigens, active immunotherapy is being investigated as a novel treatment modality for myeloma. Immunization against the clonal myeloma immunoglobulin (idiotype) elicits protective immunity in mouse models. Idiotype vaccination of myeloma patients can induce cellular immunity, albeit as yet without firm evidence for improved clinical outcome. Other tumour-associated antigens (including cancer/testis antigens) are expressed with various frequencies in myeloma. T cells with specificity for these antigens may exist in myeloma patients, and immunization trials with some of these antigens are ongoing. Future studies need to identify the best antigen, the optimal vaccine formulation and schedule, and the preferable clinical situation for vaccination with myeloma antigens. In addition, immunization of stem cell donors with myeloma antigens may improve the efficacy and outcome of allogeneic stem cell transplantation through transfer of idiotype-directed immunity to the patient, and has already shown promising clinical results.

1. Introduction

Normal plasma cells emerge from a complex selection and differentiation process of the immune system, in which affinity maturation of immunoglobulins and isotype switching are key genetic events required for antibody production. After plasma cell precursors have successfully undergone this maturation, plasma cells may be considered as cellular factories that produce high amounts of soluble, individual antibody. In this final maturation stage, plasma cells appear to be much less dependent on intercellular signals that characterize earlier stages of lymphocyte differentiation. This fact is mirrored in a relative paucity of surface immune system signalling receptors on plasma cells, including low expression levels of membrane-bound surface immunoglobulin, and HLA class II molecules.[1,2] Accordingly, few cell surface molecules have been identified as yet that are suitable targets for the development of passive immunotherapy against multiple myeloma (Chatterjee and Chakraborty, this issue). Therefore, the remarkable progress in the therapy of B-cell lymphomas achieved by combining chemotherapy with monoclonal antibodies, such as rituximab or alemtuzumab, has not been paralleled in myeloma as yet.

On the other hand, the detection of a graft-versus-myeloma effect exerted by donor-derived T lymphocytes after allogeneic stem cell transplantation (Zeiser and Finke, this issue) has demonstrated that myeloma cells are susceptible to cellular immunity. The successful induction of anti-myeloma immunity in the patient's own immune system, however, has to overcome a marked immunodeficiency of myeloma patients. The impaired immune system in myeloma is, for example, evident in a well-recognized susceptibility to infectious complications.[3] Recent evidence points to an underlying dysfunction of dendritic cells in myeloma patients.[4] In addition, a dysfunction of regulatory T cells may also impair the immune function in myeloma and its premalignant condition, monoclonal gammopathy of unknown significance (MGUS), but the precise nature of this defect remains at

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present somewhat controversial.[5,6] Furthermore, there is evidence that a reversible defect in natural killer cell function is associated with progression from premalignant gammopathy to malignant myeloma.[7] Despite these immunological dysfunctions, T cells with cytotoxic activity against premalignant cells can be detected in the bone marrow of patients with MGUS.[8] In addition, autologous T cells isolated from bone marrow exhibit preferential lysis of myeloma cells and their precursors after non-specific stimulation through CD3 and CD28.[9] These observations have renewed interest in active immunotherapy of myeloma as an adjunct to conventional or molecularly targeted pharmacotherapy.

The purpose of this review is to summarize studies to identify myeloma-associated T cell antigens, to characterize the state of the cellular immune system of patients and their potential haematopoietic stem cell donors with regard to the recognition of these antigens, and to describe preclinical models and clinical trials designed to exploit these findings for the development of active immunotherapy against multiple myeloma.

2. Active immunotherapy approaches targeting the myeloma idiotype

2.1. *The idiotype of the monoclonal immunoglobulin as a myeloma-associated antigen*

Each antibody has a unique antigen-binding site which is formed by hypervariable stretches within the variable part of the immunoglobulin polypeptide chains, the so-called "complementarity-determining regions" (CDR) I–III. The CDR III region is created during the antigen receptor gene rearrangement process, in which extensive codon deletions and insertions occur almost at random at the junctions between the rearranging variable (V), joining (J), and – in the case of Ig heavy chains – diversity (D) segments. During a germinal centre reaction, the Ig genes encoding an antigen-binding antibody are further modified by somatic hypermutation. The enormous diversity of immunoglobulins resulting from these genetic events implies that an individual antibody molecule should comprise unique epitopes that may be recognized specifically by the immune system and may hence serve as specific targets of a cellular immune response. Collectively, the unique immunological properties of any individual Ig are referred to as "idiotype".

2.2. *Preclinical models of idiotype vaccination in multiple myeloma*

Exploiting myeloma idiotype as a tumour-associated antigen was proposed over 30 years ago.[10] Induction of protective anti-tumour immunity through immunization with a myeloma idiotype has been most extensively studied in the murine plasmacytoma MOPC-315 model. In this model, weekly immunizations with tumour-derived paraprotein protects syngeneic mice against a subsequent challenge with MOPC-315 cells.[11] Vaccination-induced, antigen-specific $CD4^+$ T cells with a restricted set of T cell receptors recognize a dominant epitope of the idiotype light chain CDR III region in the context of MHC class II molecules.[12,13] Adoptive transfer of TCR-transgenic, MOPC-315-specific T cells controlled tumour growth in syngeneic mice, despite the fact that the tumour cells themselves do not express MHC class II molecules.[14] A necessary condition for tumour control was the uptake and presentation of the secreted idiotype by dendritic cells, a potent type of professional antigen-presenting cell.[15] Activation of infiltrating macrophages through IFNγ appeared to be indispensable for T cell-mediated tumour protection.[16] The Fas/FasL pathway has been identified as one of several potential effector mechanisms for elimination of MOPC-315 cells.[17] Importantly, T cell surveillance can be overwhelmed by a high tumour burden with high levels of paraprotein, leading to the deletion of idiotype-specific T cells.[18]

A substantial body of experiments has been conducted in murine idiotype vaccination models to identify the most efficient formulations and immunization routes to elicit protective and therapeutic immunity to B cell malignancies.[19] Promising formulations include the use of GM-CSF and dendritic cells, chemical coupling of idiotype protein to immunogenic carrier molecules such as keyhole limpet haemocyanin (KLH), and fusion molecules of idiotype with various immunostimulatory cytokines. Most of the studies, however, are based on non-secreting lymphoma models, where anti-idiotype antibodies appear to play a major effector role in many immunization settings. Since myelomas generally lack surface Ig expression, the findings of many studies can therefore not be directly applied to immunotherapy of myeloma.

In contrast, preclinical DNA vaccination with expression plasmids encoding myeloma idiotype in a scFv format has yielded very promising results. While vectors encoding idiotype only achieved poor tumour protection against MHC class II- and surface idiotype-negative 5T33 myeloma cells, the immune response was vastly enhanced when the scFv gene was genetically fused to a cDNA encoding fragment C of the tetanus toxin.[20] Repeated intramuscular injections of these plasmids resulted in the protection of the majority of test animals against a tumour challenge. Interestingly, since no idiotype-specific cytotoxic T cells were detectable, the effector mechanism is thought to involve cytokine release by T helper cells activated via antigen-presenting cells, possibly similar to the MOPC-315 model.

2.3. *In vitro data on idiotype-directed immunity in myeloma patients*

Antigen-specific responses, of both CD4- and CD8-positive T cells, upon *in vitro* stimulation with autologous paraprotein have been described in patients with monoclonal gammopathies.[21,22] A detailed study in one of these patients identified several CDR-derived peptide epitopes that induced IFNγ release by T cells in a MHC-restricted manner as detected by ELISPOT analysis.[23] A more comprehensive search for T cell epitopes within idiotype sequences of myeloma patients by HLA binding prediction algorithms confirmed these findings and demonstrated that most epitopes are located in the CDR II-FR III-CDR III region.[24] Induction of cytotoxic T cell activity against autologous myeloma cells could also be

shown upon stimulation with idiotype-loaded dendritic cells.[25,26]

2.4. *Clinical trials of idiotype vaccination in multiple myeloma*

Since native idiotype protein can usually be obtained from the serum of myeloma patients, vaccination trials can be relatively easily realized in multiple myeloma. Injection of paraprotein alone may lead to an increase in cellular and humoral immune responses, but appears to be insufficient to generate sustained anti-myeloma immunity.[27] When intradermal injections of paraprotein were combined with subcutaneous (s.c) administration of GM-CSF at the same site, an increase in the numbers of IFNγ- and IL-2-secreting T cells in response to idiotype was induced.[28] This response was present in $CD4^+$ and $CD8^+$ T cell subsets and could be inhibited by blockade of MHC class I molecules. Furthermore, production of idiotype-specific IgM was induced *in vivo*. However, there was no clear indication of clinical efficacy since paraprotein levels remained essentially unchanged and DTH (delayed type hypersensitivity reaction) responses to idiotype protein were not detectable. In contrast, in a trial assessing subcutaneous vaccination with KLH-coupled paraprotein and additional adjacent injections of GM-CSF in myeloma patients after high-dose chemotherapy and autologous stem cell transplantation (autoSCT), DTH reactions to the vaccine were induced in 85% of patients, but *in vitro* testing provided little evidence for specific T cell immune responses.[29,30] When patients in stage I disease were immunized with idiotype in conjunction with IL-12 +/− GM-CSF, a decrease in circulating clonal cells was detected by quantitative PCR monitoring in four of six patients.[31] Finally, intradermal immunization with uncoupled recombinant idiotype in conjunction with GM-CSF induced idiotype-specific T cell reactivity in a patient with advanced myeloma.[32]

More recently, idiotype-loaded dendritic cells (DCs) have been used by various groups as vaccines in multiple myeloma patients (Table 1), mostly in the setting of clinical remission after autoSCT.[33–39] Notable differences between the trials, which may well influence their immunological efficacy, are the source of dendritic cells, the format of the idiotype antigen (e.g. conjugated to KLH, fractionated), the route of vaccination (s.c. versus intravenous), and the use of adjuvant cytokines to enhance immunoreactivity (GM-CSF, IL-2). Although the patient characteristics and vaccine particularities preclude firm comparisons between these trials, they nevertheless have collectively shown that the induction of cellular immune responses is possible in the setting of minimal disease burden after autoSCT. However, no real evidence has been obtained in these phase I and II trials that the natural course of the disease has been altered by idiotype vaccination, and continuous efforts to improve the immunogenicity of the vaccination are ongoing.

A regulatory issue of these approaches pertains to the manufacturing of the vaccine. Almost uniformly, the idiotype has been prepared form the serum or urine of the patients through various purification steps. While this technology is fairly convenient and reliable, the resulting immunoglobulin preparation is practically always contaminated with traces

Table 1 – Clinical Trials with idiotype-presenting dendritic cells in multiple myeloma

Reference	n patients	indication	Antigen	DC type	n vaccinations, route, adjuvant cytokines	in vitro Assays
Reichardt (1999)	12	Remission after autoSCT	clonal Ig, clonal Ig-KLH	density gradient purification	2 × DC i.v., 5 × Id-KLH s.c.	2/12 Proliferation 1/3 CTL
Lim (1999)	6	active disease	clonal Ig + KLH	Mo-derived (monocyte-derived)	3 × DC i.v.	5/6 Proliferation 3/6 CTL
Cull (1999)	2	PD, SD	clonal Ig + KLH	Mo-derived	4 × DC i.v.	2/2 Proliferation 0/2 CTL
Liso (2000)	26	Remission after autoSCT	clonal Ig (12); clonal Ig - KLH (14)	density gradient purification	2 × DC i.v., 5 × Id-KLH s.c.	4/26 Proliferation
Titzer (2000)	11	active disease	proteolytic fragments of clonal Ig	$CD34^+$ -derived	1 × DC s.c., 3 × Id s.c. + GM-CSF	4/10 ELISpot
Yi (2002)	5	Remission after autoSCT	monoclonal Ig	Mo-derived	3 × s.c., IL-2 × 5d	2/5 Proliferation 4/5 ELISpot
Reichardt (2003)	12	Remission after autoSCT	clonal Ig, clonal Ig-KLH	Mo-derived	1 × DC s.c., Id-KLH s.c. + GM-SCF	2/10 Proliferation 1/10 CTL

of polyclonal immunoglobulins. Therefore, rational purity criteria for such vaccines need to be established.

Similarly, the preparation of DC vaccines needs to be standardized with respect to cell source, purity, and maturation status prior to immunization. For example, the use of bovine proteins will have to be abandoned entirely, and minimal percentages for DC (e.g., >90%) as defined by common immunophenotypic criteria have to be established. At present, a phase II trial for idiotype vaccination in myeloma patients is open for enrollment (www.ukl.uni-freiburg.de/med/med1/homede.htm).

A special aspect of active immunotherapy in multiple myeloma is the combination of allogeneic stem cell transplantation (alloSCT) with the induction of lymphoma-specific immunity in the donor's immune system. The donor immune system is presumably naïve for the patient's myeloma idiotype and therefore not tolerized or anergic. Therefore, induction of tumour-specific immunity in donors of haematopoietic stem cells for myeloma patients by idiotype immunization, followed by adoptive transfer of specific immune cells into the transplanted patient may render allogeneic SCT from a non-specific form of active immunotherapy into a tumour-specific therapy.

In the 38C13 mouse lymphoma model, mice receiving marrow from a donor immunized with 38C13 idiotype had a statistically significant survival advantage after a lethal challenge with 38C13 lymphoma cells compared to animals transplanted with control marrow.[40] When pre-immunized marrow transplantation was combined with a subsequent booster immunization, even tumour-bearing mice could evidently be cured of their disease. The protective effect was mediated by donor-derived T cells.

In a proof-of-principle report by the same group, a bone marrow donor was immunized with KLH-coupled myeloma idiotype.[41] After transplantation, an idiotype-specific, MHC class II-restricted T cell proliferation could be detected in the blood of the female stem cell recipient. The responding T cells were of donor origin as demonstrated by FISH for the Y chromosome.

More recently, results from a formal clinical trial of donor idiotype immunization were reported.[42] Five patients and their related donors received three s.c. vaccinations with idiotype (coupled to KLH at the 1st vaccination) and GM-CSF prior to alloSCT. All donors developed cellular and humoral anti-idiotype immune responses. After bone marrow transplantation, the three patients who survived longer than 30 days received 3 booster vaccinations with KLH-coupled idiotype and GM-CSF. Remarkably, these patients survived without evidence for disease recurrence for 5.5 to more than 8 years, and all had evidence for idiotype-specific immunity after alloSCT. Two trials testing this strategy further are currently recruiting patients (www.clinicaltrials.gov).

In order to avoid immunization of the healthy donor, attempts have been made to generate myeloma idiotype-specific donor immunity through *in vitro* stimulation of donor T cells with monocyte-derived, idiotype-presenting DC.[43] Implementation of this approach would permit to extend the principle of transfer of tumour-specific immunity to the vast pool of unrelated stem cell donors for alloSCT.

3. Cancer/testis antigens as potential targets for immunotherapy of multiple myeloma

Cancer/testis (C/T) genes are physiologically expressed in testis and placenta trophoblast only and have a potential role in cell cycle and apoptosis. C/T gene expression is reactivated by hypomethylation of their promoter regions in malignant tumours, most prominently in malignant melanoma.[44] Since testis is an immunologically privileged tissue lacking HLA expression, CT genes act as tumour-specific antigens. HLA class I-restricted cytotoxic T cells with specificity for peptide epitopes derived from different C/T genes have been identified in a few cancer patients with a rather unusual and favourable disease course.[45] Active immunotherapy directed at C/T antigens is currently being investigated in various cancer types with particular emphasis in melanoma.

Several groups have described that C/T genes are also expressed by myeloma cells,[46–52] albeit with somewhat lower frequency than in melanoma (Table 2). Depending on the patient population and method used to detect C/T gene expression, there appears to be a trend towards higher likelihood of expression with advanced stage[47,49] and presence of cytogenetic abnormalities,[50] both representing adverse prognostic factors in myeloma. Due to this fact, C/T antigens may be favourable to idiotype, since myelomas may lose expression of Ig genes during disease progression. C/T Ag-specific T cells can be detected in the blood of myeloma patients and appear to be functionally competent.[50,53] Several clinical trials for vaccination with MAGE-A3 and NY-ESO-1-derived antigenic peptides are currently open for enrollment of patients with multiple myeloma (details under www.clinicaltrials.gov and www.kimt.de).

4. Lineage-restricted and universal antigens as potential targets for immunotherapy of multiple myeloma

MUC1 is an epithelial mucin that is physiologically highly glycosylated. Since the molecule is often expressed but severely underglycosylated on malignant cells, it may be recognized by cytotoxic T cells (CTLs) in a MHC-unrestricted manner.[54] This effect has also been shown in myeloma.[55] Furthermore, HLA class I-restricted peptide target epitopes have also been identified within the MUC1 sequence, and the majority of myelomas appears to express and present these epitopes to T cells.[56] MUC1 peptide-specific, functionally competent $CD8^+$ T cells are detectable in a sizable fraction of the blood and bone marrow of myeloma patients.[57]

WT1 is a zinc finger transcription factor with overexpression in myeloid malignancies.[58] WT1-specific $CD8^+$ T cells can be found in patients with acute myeloid leukaemia.[59] While WT1 is also frequently expressed, albeit at lower level, in lymphoid malignancies, myeloma cells may be efficiently recognized and lysed by WT1-specific CTL.[60]

CD317/HM1.24, a cell surface protein involved in cell signalling,[61] is another potential tumour-associated antigen overexpressed in multiple myeloma.[62,63] HM1.24-specific CTL can be induced in healthy volunteers and multiple myeloma patients.[64–66]

Table 2 – Studies analyzing the expression of cancer/testis antigens in MGUS and multiple myeloma

C/T Gene	Patient Group	Sample size	Method	% expression	Reference
MAGE-A1	stage III	29	RT-PCR	28	van Baren (1999)
	n.g.	21	RT-PCR	76	Pellat-Deceunynck (2000)
	stage I-III	29	IHC, FCM	24	Dhodapkar (2003)
MAGE-A2	stage III	29	RT-PCR	17	van Baren (1999)
	n.g.	21	RT-PCR	57	Pellat-Deceunynck (2000)
MAGE-A3	stage III	29	RT-PCR	28	van Baren (1999)
	stage I-III	29	IHC, FCM	48	Dhodapkar (2003)
MAGE-A3/6	MGUS	15	IHC	40	Jungbluth (2005)
	stage III	33	IHC	70	Jungbluth (2005)
MAGE-A4	stage III	29	RT-PCR	17	van Baren (1999)
	stage I-III	29	IHC, FCM	21	Dhodapkar (2003)
MAGE-A6	stage III	29	RT-PCR	31	van Baren (1999)
MAGE-A10	stage III	29	RT-PCR	7	van Baren (1999)
MAGE-A12	stage III	29	RT-PCR	14	van Baren (1999)
MAGE-C1/CT-7	stage I-III	29	IHC, FCM	79	Dhodapkar (2003)
	MGUS	15	IHC	13	Jungbluth (2005)
	stage III	33	IHC	82	Jungbluth (2005)
BAGE-1	stage III	29	RT-PCR	14	van Baren (1999)
	n.g.	21	RT-PCR	5	Pellat-Deceunynck (2000)
GAGE-1/2	stage III	29	RT-PCR	41	van Baren (1999)
	n.g.	21	RT-PCR	33	Pellat-Deceunynck (2000)
GAGE-3-6	stage III	29	RT-PCR	41	van Baren (1999)
LAGE-1	stage III	29	RT-PCR	52	van Baren (1999)
NY-ESO-1	stage III	29	RT-PCR	31	van Baren (1999)
	primary Dx, normal karyotype	126	Microarray, IHC	31	van Rhee (2005)
	primary Dx, abnormal karyotype	35	Microarray, IHC	60	van Rhee (2005)
	relapse, normal karyotype	28	Microarray, IHC	61	van Rhee (2005)
	relapse, abnormal karyotype	27	Microarray, IHC	100	van Rhee (2005)
	stage I-III	29	IHC, FCM	27	Dhodapkar (2003)
	MGUS	15	IHC	0	Jungbluth (2005)
	stage III	33	IHC	21	Jungbluth (2005)
PRAME	stage III	29	RT-PCR	48	van Baren (1999)
	n.g.	21	RT-PCR	52	Pellat-Deceunynck (2000)
RAGE-1	n.g.	21	RT-PCR	0	Pellat-Deceunynck (2000)
Sp17	n.g.	47	RT-PCR, Western blot	26	Lim (2001)
Span-X1	n.g.	30	RT-PCR	20	Wang (2003)

n.g. = not given; IHC = immunhistochemical analysis, FCM = flow cytometric analysis.

Since the expression of these genes is not restricted to tumours, it remains to be established whether C/T antigens or lineage-associated antigens are superior targets for anti-myeloma immunotherapy. At present, only one clinical trial investigating MUC1 vaccination has been registered (www.clinicaltrials.gov).

5. Cell-based myeloma vaccines

Instead of vaccinating myeloma patients against defined tumour antigens, an alternative principle aims at stimulating the immune system with the entirety of the myeloma cell's antigens. Such approaches may be implemented by using tumour cell lysates or apoptotic tumour cells as a source of antigens. Indeed, stimulation of T cells from the peripheral blood or bone marrow with autologous dendritic cells that had been co-incubated with purified, irradiated myeloma cells may give rise to T cell lines with specific IFNγ production and lytic activity of primary autologous tumour cells.[67] In this approach, presentation of antigens from myeloma cell lines by dendritic cells is greatly enhanced by coating of myeloma cells with a specific antibody such as anti-CD138.[68] Similar results with induction of specific, mostly perforin-dependent cytotoxic T cell activity against autologous myeloma cells has also been reported when dendritic cells were loaded with myeloma cells lysed by repetitive freeze-thaw cycles.[69] Interestingly, the candidate myeloma antigens, idiotype and NY-ESO-1, appeared to be some of the recognized targets in at least some of the patients analyzed.[68,69] In a direct comparison, irradiated, apoptotic tumour cells appeared to be a superior source of antigen compared to tumour lysates for dendritic cell-mediated T cell stimulation.[70]

These results demonstrate that dendritic cells presenting antigens from the myeloma cell proteome hold promise as autologous tumour vaccines or as powerful tools to generate tumour-specific effector T cells for adoptive transfer. As is the case in any other patient-individual approach, regulatory issues such as tumour cell purity and specifications need to

be solved prior to large-scale clinical testing. One solution to this problem would be the use of standardized myeloma cell lines as common antigen sources. Whether this approach would indeed induce T cell responses with optimal or at least sufficient specificity in the majority of patients, however, needs to be verified in a sizeable patient cohort.

6. Conclusions

Since multiple myeloma continues to represent an incurable disease with fatal outcome in the majority of patients in advanced stages despite rapid development of new pharmacological compounds, the exploration of novel therapeutic modalities such as immunotherapy should definitively be pursued. In addition to the classical antigen of malignancies of the B cell lineage, i.e. the clonal idiotype, a number of different tumour-associated proteins that may act as T cell antigens have recently been identified. The combined evidence from idiotype vaccination studies indicates that cellular immune responses can be induced successfully in myeloma patients, but that the immunological efficacy is as yet insufficient to provide a clearly recognizable benefit for the vaccinated patients. Therefore, idiotype vaccination strategies need to be improved with regard to their immunogenicity and duration of therapy, and need to be explored in different and more favourable clinical situations, such as MGUS with high risk for progression to overt myeloma. In parallel, the existing experience with idiotype vaccination can serve as a basis to test and to compare vaccination against a variety of novel candidate myeloma antigens. C/T antigens are the best studied examples within this category, although their exploitation requires profiling of the C/T antigen expression pattern in every individual patient. Additional antigens continue to be identified in myeloma and call for systematic assessment and comparisons to identify the most promising candidates for clinical trials.

Perhaps the most interesting field for active immunotherapy in myeloma lies in the combination with allogeneic stem cell transplantation. This setting offers the advantage of an immune system that is unaffected by potential negative influences exerted by the tumour on the immune system. Transfer of tumour antigen-specific immunity from the donor to the myeloma patient may help to enhance the immunological efficacy of allogeneic SCT and to separate graft-versus-myeloma from graft-versus-host activity. The most crucial question to develop this concept further is whether the donor has to be immunized personally or whether efficacious, specific antitumour immunity can be induced *ex vivo* or in the transplanted patient.

Conflict of interest statement

None declared.

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