Idiotype-specific immunotherapy in multiple myeloma: suggestions for future directions of research

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Multiple myeloma (MM) remains a difficult-to-cure cancer and less than 20% of patients achieve long-term survival irrespective of the treatment delivered, including high-dose chemotherapy. Thus, new treatment modalities are urgently needed. Myeloma cells produce a monoclonal immunoglobulin (Ig) which is a truly tumor-specific antigen. The tumor-specific antigenic determinants are localized in the variable regions of the monoclonal Ig and are termed idiotopes (Id). Id-vaccination, i.e., vaccination with the autologous monoclonal Ig, has been performed in MM patients in order to elicit tumor-specific immune responses and possibly elimination of myeloma cells. However, clinical trials have not given the promising results obtained in mice. This review focuses on tolerance mechanisms that might hinder Id-specific immune responses in MM patients. New strategies for Id vaccination in MM are discussed.

Key words: T cells, idiotype, tolerance, immunosurveillance, cancer.

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regions generated by clonal rearrangements of V(D)J gene segments and by somatic hypermutation. These V-region antigenic determinants are called idiotopes, and the sum of the idiotopes represents the idiootype (Id) of the monoclonal Ig. Id expressed by the monoclonal Ig in MM has distinct advantages as a tumor-specific antigen. Firstly, consistent with being derived from post-germinal B cells, myeloma cells usually contain numerous somatic mutations in their rearranged Ig V(D)J genes. Secondly, the somatic mutation process appears to have stopped in MM so that cells do not acquire further amino acid replacements in their V regions. Thus, since myeloma protein V regions do not change over time, Id is a stable tumor-specific marker. Id also has certain practical advantages as a tumor-specific antigen. Firstly, because assembled V(D)J gene segments of Ig heavy (H) and light (L) chains may be relatively easily amplified and sequenced from bone marrow samples, tailor-made DNA-based Id vaccines can be constructed for each patient without too much effort. Secondly, because monoclonal Ig can easily be purified from patient serum or transfected cells, protein-based Id vaccines can readily be prepared.

**Id as a tumor-specific target for immune attack: basic immunological mechanisms**

Prophylactic Id vaccination of mice protects against tumor challenge with Id-positive myeloma, as demonstrated by Eisen and colleagues and confirmed by a number of other investigators. However, in order to design effective strategies for Id vaccines, it is crucial to understand the molecular and cellular mechanisms by which Id-specific immune responses are capable of eradicating myeloma cells. Experiments in mice have shown that Id-specific antibodies do not play a major role in tumor eradiation, the reason being that the large quantities of soluble monoclonal Ig secreted by myeloma cells block Id-specific antibodies before they can reach the surface of myeloma cells. Even if Id-specific antibodies could escape peripheral blockade and reach the vicinity of myeloma cells, they are unlikely to be effective since myeloma cells usually express little or no surface Ig (Figure 1A). Id-specific CD8+ T cells could have a role in immnosurveillance because MM cells usually express major histocompatibility complex (MHC) class I molecules. Moreover, studies in mice have demonstrated that myeloma cells process their endogenous Ig and present Id peptides on their MHC class I molecules to CD8+ T cells (Figure 1B). However, there is not yet much information on the role of Id-specific CD8+ T cells in vivo in MM. Id-specific CD4+ T cells have been considered unlikely to play a role in MM since myeloma cells usually do not express many MHC class II molecules. However, extensive studies in mice, reviewed by Corthay et al., have shown that Id-specific CD4+ T cells clearly play a role in eradication of MHC class II-negative MOPC315 myeloma cells. As a mechanism, it was demonstrated that monoclonal Ig secreted by the tumor was endocytosed and processed by antigen-presenting cells in the draining lymph node and within the tumor. Such antigen-presenting cells presented Id-peptides on their MHC class II molecules to Id-specific CD4+ T cells of the Th1 type. The activated tumor-infiltrating Id-specific CD4+ T cells produced interferon-γ that stimulated macrophages so that these latter cells became tumoricidal and killed myeloma cells (Figure 1C).

**Evidence for naturally occurring Id-specific T-cell responses in MM patients**

The observation that Id-specific CD4+ T cells protect mice against MM raises the question as to whether Id-specific T cells have relevance in human disease. MM patients have perturbations of their αβ T-cell receptor
(TCR) repertoire with clonal and oligoclonal expansions particularly in the CD8+ populations.24-27 Dendritic cells, too, have been shown to be dysfunctional.64-66 These abnormalities are expected to generally lower the efficiency of T-cell responses in MM patients. Despite this, even in the absence of Id vaccination, low frequencies of Id-specific T cells have been detected by adhesion, proliferation, and cytokine secretion (ELISPOT) assays in patients with monoclonal gammopathy of undetermined significance (MGUS) and MM.46-50 These low frequency Id-specific T cells do not appear to be related to the clonally or oligoclonally expanded T cells. Such Id-specific T cells responded to synthetic peptides corresponding to the complementarity determining regions (CDR) of both H and L chains of the monoclonal Ig.46-50 Id-specific T cells appeared to be more frequent among CD4+ cells than among CD8+ cells. While Th1 cells that produced interferon-γ dominated in the early stages of the disease, Th2 cells that produced interleukin-4 dominated with disease progression.53 Id-specific cytotoxic T-cell lines with the capacity to kill autologous primary myeloma cells were also generated in vitro.55,56 The cytotoxic T-cell lines consisted of both CD4+59 and CD8+60 T cells. Killing of MM was in one report solely MHC class I-restricted58 while in the other report both class I- and class II-restriction was observed.59 Collectively, these results suggest that Id-specific T cells can naturally occur in MGUS and MM patients and can be involved in controlling the progression of the disease. T cells with other specificities may also play a role in both MGUS and MM.24

However, an alternative interpretation is that in MM patients most high avidity Id-specific T cells have been deleted and that only low avidity T cells remain (see below). Although such low avidity Id-specific T cells are detected by sensitive techniques in vitro, their significance in vivo is not known. It is, in fact, difficult to test whether high avidity Id-specific T cells are tolerated in MM patients, simply because the Id-specific repertoire prior to disease is unknown.

**Id-specific vaccination in MM patients**

Since Id vaccination confers protection against MM in mice,27 it has been important to investigate the effects of Id vaccination in MM patients. A number of different strategies for Id-vaccination have been employed. In some studies, untreated patients with early stage MM were immunized with autologous alum-precipitated myeloma protein, either with48 or without25 granulocyte-macrophage colony-stimulating factor (GM-CSF). In other studies, Id vaccination was performed with conjugates of Id-keyhole-limpet hemocyanin (Id-KLH) in association with GM-CSF or interleukin-2,24 or with Id-pulsed dendritic cells.56 Some of these studies were performed in untreated patients while others were performed after high-dose chemotherapy and stem cell transplantation. Id-specific T- and B-cell responses were detected with variable frequency, but clinical responses were unsatisfactory and not correlated with the induction of tumor-specific immune responses. Thus, it is too early to say whether Id-vaccination and elicitation of Id-specific immune responses might improve the prognosis of MM patients.57-59 It should be emphasized that the same vaccine formulations, i.e., Id/KLH conjugates or dendritic cell-based Id vaccines, induced Id-specific immune responses with higher frequency in patients with B-cell lymphoma, with clear evidence of tumor burden reduction and/or improvement of clinical outcome.60-62 The effectiveness in B-cell lymphomas is a proof of principle of the validity of Id as a tumor-specific target for immune attack, but it also strongly suggests that the immune competence status and mechanisms of Id tolerance play a much more important role in MM than in lymphoma, as discussed below.

**The issue of T-cell tolerance to Id in MM**

If Id vaccination is to be useful in MM patients, T cells have to be able to respond. This is an issue that has been largely ignored despite theoretical and experimental evidence of its importance. First, in order to elicit any T-cell responses at all, Id peptides derived from the myeloma must be able to bind the MHC molecules of the individual,57-59 and this might often not be the case. Second, even if Id peptides are able to bind MHC molecules of the individual, one should consider that T cells can either respond, or become tolerant. The tolerance issue can be further divided into two scenarios: (i) tolerance to germline encoded Id peptides prior to disease, and (ii) emergence of tolerance to Id peptides with disease development.

As to the first issue, a number of basic immunological experiments strongly suggest that CD4+ T cells are tolerant to germline encoded Id peptides.60-67 In part due to deletion in the thymus.71,72 Thus, Id-specific CD4+ T cells should selectively focus on Id peptides dependent on somatic mutation of rearranged V(D)J in the myeloma cells. This might not be a major problem in MM, since V gene regions are usually heavily mutated. As to the second issue, which is of the utmost importance, it appears that Id-specific CD4+ T cells become tolerant as MM disease progresses. This evidence was obtained in Id-specific TCR-trangenic mice challenged with such high amounts of MÔPC315 MM cells that the resistance conferred by Id-specific CD4+ T cells was overcome.75,76 Such experiments demonstrated that if T cells failed to eliminate myeloma cells upon their initial encounter, T-cell tolerance ensued and MM tumors progressed.75,76 More specifically, Id-specific CD4+ T cells were progressively deleted once the serum myeloma protein concentration exceeded 50 µg/mL. Deletional tolerance was evident not only in the thymus, but also in peripheral lymphoid organs, and even in the MM tumor itself.77 Based on this model, one would expect that MM patients in whom the myeloma protein concentration is much higher than 50 µg/mL at diagnosis would have no functional Id-specific CD4+ cells left due to tolerance development prior to disease detection. Even individuals with MGUS, whose monoclonal component serum levels are typically less than 5 mg/mL in the absence of any symptoms, may have undergone the same tolerance process as that in MM patients.

Close to nothing is known about the tolerance of Id-specific CD8+ T cells. However, it has been argued that CD8+ T-cell responses to influenza hemagglutinin are
reduced due to tolerance to the cross-reactive V\textsubscript{i}\textsubscript{49-58} sequence of the V\textsubscript{i} gene segment used in the MOPC21 plasmacytoma; this Ig V-region sequence differs by only one amino acid from the relevant hemagglutinin sequence.\textsuperscript{70-73}

**Suggestions for future directions of research**

**Better characterization of Id-specific T-cell responses in humans**

As referenced above, many investigators have described Id-specific T cells occurring naturally, as well as after Id immunization, in MM patients. These findings appear to contradict the studies on T-cell tolerance done in two different Id-specific TCR-transgenic strains of mice in which tolerance development can be easily monitored.\textsuperscript{74-76} To reconcile these seemingly contradictory findings, it is reasonable to suggest that Id-specific T cells should be better characterized in humans. Preferably, Id-specific T cells should be cloned, and their specificity for Id should be documented both with synthetic peptides as well as with the complete monoclonal Ig. The MHC restriction elements should also be defined. These requirements are not unreasonable as they are usually met by T-cell immunologists working with other antigens in humans. Finally, the avidity of Id-specific T cells should be investigated, e.g. by establishing dose-response curves. It is entirely possible that a more detailed investigation in MM patients will actually reveal that there is a substantial degree of T-cell tolerance to Id in MM as in TCR-transgenic mouse models.

Besides a better characterization of Id-specific T cells, it is important to investigate the role of inhibitory mechanisms such as those mediated by regulatory T cells (Tregs). Relief of inhibitory signals mediated by Tregs has been shown to improve the potency of vaccine-induced antitumor immune responses.\textsuperscript{77} Thus, in the MOPC315 plasmacytoma model, it was observed more than 20 years ago that large subcutaneous tumors could be cured by either low (15 mg/kg) or high (300 mg/kg) doses of cyclophosphamide. However, only mice treated with low dose cyclophosphamide were able to reject a lethal challenge with MOPC315 cells following chemotherapy, strongly suggesting the establishment of antitumor immunity.\textsuperscript{77} Since Tregs are sensitive to low dose cyclophosphamide,\textsuperscript{78} these data would suggest, though indirectly, their involvement in control of immune responses against myeloma. Very little is known about Tregs in human MM, and whether they can influence Id-specific immune responses. Recently, two conflicting reports have been published, one describing Treg dysfunction,\textsuperscript{79} the other suggesting an increased frequency and normal immunosuppressive function of Tregs isolated from patients.\textsuperscript{80}

**The tolerance problem: should only patients in complete remission be vaccinated?**

If there is actually a substantial degree of T-cell tolerance to Id in MM patients, it should be of overriding importance to reverse T-cell tolerance prior to Id-vaccination. As judged from the results in an Id-specific TCR-transgenic model, reversal might be obtained once the serum myeloma protein concentration has dropped below <50 µg/mL. However, this estimate is based on results obtained in a single Id-specific TCR transgenic model.\textsuperscript{74-76} It might well be that in patients with a polyclonal Id-specific TCR repertoire, the serum concentration required for relief of tolerance could vary for individual Id-specific T cells. Likewise, the monoclonal Ig concentration needed for induction of T-cell tolerance could differ between mice and men, and even between individual MM patients. Stem cell transplantation is considered the treatment of choice for MM patients up to the age of 65 years old.\textsuperscript{103-105} The complete remission rate varies from 20 to 40%, depending on the criteria used to define complete remission. Recently, it has been proposed that the monoclonal Ig must no longer be detectable by immunofixation, which has a sensitivity level of about 50-200 µg/mL of serum myeloma protein, in order for the patient to qualify as having complete remission.\textsuperscript{82} However, since the amount of residual circulating monoclonal Ig in immunofixation-negative patients in complete remission has not been systematically examined, it is unknown whether such patients really have myeloma protein concentrations below the critical <50 µg/mL threshold. Thus, more sensitive techniques should be developed to determine the degree of complete remission, e.g. patient-specific ELISA and PCR methods. Patients in molecular complete remission, as defined by PCR methods based on tumor-specific V(D)J PCR primers, may indeed have very low amounts, or no, circulating monoclonal Ig, but these patients probably represent a very small minority after stem cell transplantation. Even if the serum levels of the monoclonal Ig do not fall below the 50 µg/mL threshold during remission, it might still be advisable to vaccinate at a time-point when the monoclonal Ig concentration is low or very low. This suggestion is based on experimental data indicating that the higher the antigen concentration, the more profound the T cell tolerance.\textsuperscript{75,76,85} Thus, once the monoclonal Ig concentration has been reduced, low avidity Id-specific T cells could regain some of their Id-responsiveness.

**The TCR repertoire and antigen-presenting cell function post-transplantation**

In addition to obtaining a complete remission, patients undergoing Id vaccination would need to educate new Id-specific T cells in the thymus from committed thymocyte precursors. It will therefore be a challenge to find the best time-point after stem cell transplantation to vaccinate: (i) monoclonal Ig concentration should be at its nadir while (ii) new antigen-presenting cells (such as dendritic cells) and (iii) a new T-cell repertoire should have emerged. The second requirement could be a problem since MM patients have been reported to have quantitatively and qualitatively deficient dendritic cells. However, sufficient dendritic cells have been obtained from MM patients to perform Id-vaccination after stem cell transplantation.\textsuperscript{57-62} As concerns the qualitative defects, one signaling pathway leading to dendritic cells dysfunction following exposure to MM cells, or their conditioning culture medium,
has recently been identified. Thus, ex vivo generated dendritic cells treated with specific inhibitors of p38 mitogen activated protein kinase (MAPK) regained full functionality and established Id-specific immunity in mice. The third requirement mentioned above, namely development of a new T-cell repertoire, might be difficult to fulfill as the T-cell receptor repertoire has been reported to be severely and long-lastingly altered in MM patients both before and after stem cell transplantation. Development of a new T-cell repertoire after transplantation could be a particular problem in MM patients, given their advanced age and thymic involution.

A recent study has shown that the immune competence of MM patients can be restored following high-dose chemotherapy and autologous stem cell transplantation by a combination of vaccination and adoptive T-cell therapy. Patients vaccinated against Streptococcus pneumoniae both before T-cell harvest and after adoptive T-cell transfer shortly following transplantation had improved immune reconstitution. However, the relevance of such results to vaccination of MM patients is not straightforward. Firstly, vaccination to prime T cells before harvest might not be successful because of tolerance, due to a high tumor burden prior to the high-dose chemotherapy. Secondly, Id-specific T cells deleted by myeloma progression would not be expected to reappear following autologous T-cell transfer.

The possibility of inducing complete remission with a combination of targeted therapy and conventional drugs is emerging as an alternative to stem cell transplantation. For instance, the association of thalidomide with melphalan and prednisone (MPT regimen) induces a complete remission rate comparable to that achievable by autologous stem cell transplantation. It is currently under investigation whether the remission status achieved by MPT or other regimens with immunomodulatory drugs such as revlimid or bortezomib preserves the immune competence status of MM patients better than stem cell transplantation does. It should be pointed out that allogeneic stem cell transplantation has the potential advantages of providing recipients with a non-tolerized T-cell repertoire. In addition, donor-derived, fully functional dendritic cells could be used for vaccination. The latter strategy has recently been tested in a pilot clinical trial.

**Id vaccines and methods of delivery**

There are many different approaches to Id-vaccination. Since the monoclonal Ig can readily be purified from serum prior to cytoreductive therapy, protein-based vaccines have been widely used in clinical trials. Id has been conjugated to carrier proteins such as KLH, and delivered in the presence of adjuvants such as alum, GM-CSF, or interleukin-12, or delivered by dendritic cells alone or with the same adjuvants. As an alternative, it is relatively easy to amplify rearranged V(D)J genes from myeloma cells and produce tailor-made Id-vaccines in DNA format. Injection of Ig-genes as naked plasmids or Id-encoding recombinant adenovirus induced anti-Id antibody responses and tumor protection in mice. Moreover, monoclonal antibodies or single chain Fv have been genetically conjugated to GM-CSF, chemokines, CD40L, tetanus toxin fragment C, and interleukin-1β, and used as protein vaccines or DNA vaccines for vaccination against myelomas and B-cell lymphomas in mice. In the years to come, novel innovative Id-containing molecules, and more efficient means of DNA vaccination, such as electroporation, are likely to further boost this approach. For example, novel bivalent molecules (vaccibodies) that target antigen-presenting cells via antigen-presenting cell-specific single chain Fv for efficient delivery of idiotypic single chain Fv have been constructed. When vaccibodies were delivered as an intramuscular DNA vaccine combined with electroporation, antigen-presenting cells in draining lymph nodes became Id-primed and stimulated Id-specific CD4+ T cells. Vaccinated mice mounted an Id-specific immune response and resisted a challenge with MOPC315 tumor. The vaccibody technology has recently also been applied to MM patients (Frøyland M, Bogen B, unpublished data).

**Should all patients be Id-vaccinated, regardless of V(D)J sequences of the myeloma protein?**

If V(D)J sequences do not contain peptide binding motifs for MHC molecules of the individual, no Id-specific T-cell responses can be expected. In this case, immunization of patients with their own monoclonal Ig would be futile. Likewise, due to T-cell tolerance, only Id peptides expressing somatic mutations or N-region diversity are expected to be immunogenic, but only in patients with very good complete remission. Thus, Ig-sequencing, HLA typing, and analyses of V regions for mutations and peptide-binding motifs are important prerequisites to increase the chances of successful Id vaccination.

**Id-specific T-cell therapy?**

Since Id-specific T cells can be tolerized or display low avidity as a consequence of long-term exposure to myeloma cells, an alternative strategy is the transfer of allogeneic Id-specific T cells concomitantly with the allotransplantation procedure. If generated with an appropriate vaccine formulation in a healthy immunocompetent donor, it is theoretically possible to generate high avidity Id-specific T cells. The requisite tumor-specificity of Id makes this antigen an ideal candidate for normal donor immunization. This approach will circumvent both Id tolerance and the disrupted T-cell receptor repertoire of MM patients. When the stem cell donor is immunized against the recipient’s Id, anti-Id humoral and cellular immunity is expected to be transferred with the graft. Although patient series are very small, results are promising and clinical trials are ongoing.

Allogeneic transplantation is also an ideal platform to test the efficacy of Id-specific immunomanipulated donor lymphocyte infusion. This suggestion is based on the observation that donor lymphocyte infusion has an effect in MM. However, because the effect seems to be mediated by alloreactive T cells, it has been difficult to separate graft-versus-myeloma and graft-versus-host effects. If highly tumor-reactive Id-specific T cells could
be transferred, the problem of graft-versus-host disease might be reduced while retaining the graft-versus-myeloma effect. Indeed, Id-specific T cells for transfer could be obtained by immunizing related donors, followed by in vitro expansion and enrichment. To avoid any potential risk associated with the exposure of a healthy donor to a cancer product, which might be ethically acceptable in the case of related,11 but not of unrelated, donors, in vitro priming and education of allogeneic donor T cells should also be considered. There is published evidence suggesting the feasibility of this approach.12 However, whether infusion of Id-specific T cells has any clinical effect is not known. The finding that transfer of Id-specific CD4+ T cells13 or an Id-specific T-cell line14 could cure mice of previously injected Id’ B lymphoma cells suggest that this could be a valuable strategy in MM.

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