

Idiotype Vaccination Strategies in Myeloma: How to Overcome a Dysfunctional Immune System

□□ Commentary on Hansson et al., p. 1503

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In this issue of *Clinical Cancer Research*, Hansson et al. vaccinated 28 patients (IgG myeloma, Salmon-Durie stages I and II) with autologous myeloma idiotype protein and assessed the effect of adding either granulocyte macrophage colony-stimulating factor (GM-CSF) alone or the combination of GM-CSF and interleukin 12 (IL-12) during the vaccination program (1). Idiotype-specific T-cell responses as measured by proliferation and ELISPOT assays, and delayed-type hypersensitivity reactions were more frequent in patients receiving GM-CSF and IL-12 compared with those treated with IL-12 alone (85% versus 33%). The median time to myeloma progression was 108 weeks in immunologic responders compared with 26 weeks for nonresponders, which provides the first tentative evidence that idiotype vaccination in myeloma may translate into clinical benefit. Immunologic nonresponse was associated with an increase in CD4⁺/CD25⁺/*Foxp3*⁺ cells (T_{reg} cells).

The immunogenicity of the idiotype protein in myeloma has been investigated for more than three decades (2). Vaccination with the idiotype protein is attractive because it provides for multiple patient-specific tumor epitopes which can be readily purified from the peripheral blood of patients with myeloma. The idiotype protein can induce humoral immunity, however, in contrast to lymphoma, myeloma cells do not express the IgG idiotype on the cell surface, and hence, the contribution of idiotype antibodies to any vaccine-induced clinical responses in myeloma is unclear. The immunogenic peptides contained in idiotypes are mostly derived from the variable CDRI, II, and III regions rather than the framework region of the IgG molecule inducing CD4⁺ and CD8⁺ T cell immune responses (3, 4). Idiotype-pulsed dendritic cells (DC) can induce autologous idiotype-specific T cells from patients with myeloma, which have the ability to kill primary myeloma cells. This proves that idiotypes are indeed naturally processed and presented by malignant plasma cells (5). Cytolysis of myeloma cells is mostly HLA class I-restricted and mediated via the perforin/granzyme mechanism (5).

Naturally occurring idiotype-specific T cells are present in monoclonal gammopathy of underdetermined significance and multiple myeloma, and were also detected in the present study. However, in advanced myeloma, the T cell responses may be shifted to type 2 inflammatory cellular responses (6, 7). This pool of preexisting idiotype-specific T cells could potentially be expanded by vaccination with idiotype proteins, but the

functional activity of these T cells is a matter of debate. Active immunization in experimental animal models provides protection against challenge with myeloma tumor cell lines (2, 8). However, results in human clinical trials with idiotype vaccination have thus far been disappointing. Immunologic responses occur in <50% of patients and clinical responses are rare (9). Perhaps this is because idiotype proteins are weak immunogens and induce only weak to intermediate affinity T cells. There have been attempts to enhance immune responses by linking idiotypes to keyhole limpet hemocyanin or tetanus toxoid in order to recruit helper T cells (10). Others have added GM-CSF at the site of injection to attract DCs and promote the processing and presentation of idiotype antigens (11). GM-CSF seems to be a critical component of vaccination regimens in lymphoma, which have clinical efficacy (12, 13).

Clinical trials with *ex vivo* idiotype-pulsed DCs have not been successful either, which in part, can be explained by the use of immature DCs and/or the i.v. administration of DCs (14). In addition, vaccination with immature DCs may favor the induction of Tr1-like regulatory T cells (15, 16). In patients with myeloma, DCs are functionally abnormal *in vivo* and exhibit an immature phenotype with reduced expression of the costimulatory molecule B7.1 (17). These DCs have a reduced ability to stimulate antigen-specific T cells and present patient-specific idiotype proteins to autologous T cells (18). Excess secretion of transforming growth factor β , IL-6, and IL-10 by myeloma cells and/or the myeloma microenvironment are responsible for these abnormalities (17, 18). Transforming growth factor β and IL-10 can induce tolerance of antigen-specific T cells and skew the immune response to a type 2 T-cell response. IL-6 is also an autocrine and paracrine growth factor for myeloma cells and inhibits the development of DCs from CD34⁺ progenitor cells (18). The number of high-potency DCs is lower with more advanced myeloma disease (18, 19). The level of β 2 microglobulin reflects tumor burden and is a poor prognostic factor in the International Staging System. β 2 Microglobulin also has detrimental effects on DC function and reduces IL-12 production by monocyte-derived DCs. β 2 Microglobulin reduces the expression of costimulatory and adhesion molecules by DCs and negatively affects the induction of T cell responses (20). Furthermore, it has recently been reported that DCs may directly support the clonogenic growth of primary myeloma cells (21). Taken together, these observations emphasize the ability of myeloma to dysregulate the function of *ex vivo* and *in vivo* generated DCs and helps to explain the poor clinical results of idiotype vaccination trials.

It is of interest to note that in the study by Hansson et al., the number of baseline CD4⁺/CD25⁺/*Foxp3*⁺ T_{reg} cells in immunologic responders was lower compared with nonresponders. In two-thirds of the immune responders, the immune response

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disappeared after the induction phase, which coincided with an increase in the number of T_{reg} cells. It is now well established that an increased number of T_{reg} cells can be found in a number of malignancies and that the presence of T_{reg} cells is associated with a poor prognosis (22). Expansion of these T_{reg} cells is at least, in part, antigen-driven and can be amplified by therapeutic vaccination (23). In monoclonal gammopathy of underdetermined significance and myeloma, peripheral expansion of functional T_{reg} (naïve, central memory, and effector memory T_{reg} cells) has recently been reported (24, 25). DCs fully matured with proinflammatory cytokines expand T_{reg} cells *in vivo* in patients with myeloma (24, 25). These T_{reg} cells are functionally active and suppress T cell proliferation. T_{reg} cell formation in myeloma is DC/T cell contact-dependent, involves the B7.1 and B7.2 molecules, and is not related to IL-10 or transforming growth factor β secretion, all features associated with classic thymic-dependent T_{reg} . In addition, it has been shown that T_{reg} cells can develop from the CD25-negative cell population, suggesting that the proposed elimination of the CD25-positive population may not always be effective in promoting vaccine-induced T cell responses (26). Transforming growth factor β , present in abundance in myeloma, could contribute to the induction of T_{reg} cells (27).

The expansion of T_{reg} cells described by Hansson et al. clearly adds a different layer of complexity to idiotype vaccination in myeloma. It seems that immature DCs can induce Tr1-like regulatory cells, whereas fully mature DCs promote the induction of T_{reg} cells. Fully mature DCs are efficient in inducing both T effector cells and T_{reg} cells with the immunosuppressive action of T_{reg} cells being dominant (23). It is clear that the outcome of vaccine-induced tumor-specific T cell responses may depend on the strength of the opposing effects of T_{reg} cells and T effectors, and that optimizing the balance of T effectors versus T_{reg} cells is pivotal. Certainly, in all future trials, the potential induction of T_{reg} cells needs to be carefully monitored.

It is of interest that in the study by Hansson et al., more immunologic responses were seen with the combination of IL-12 and GM-CSF, and the median time to progression in the immunologic responders was 108 weeks versus 26 weeks in nonresponders. It should be recognized, however, that the number of patients in the study was small and that it cannot be excluded that the ability to respond to the idiotype protein might be due to the differing disease characteristics in the two cohorts. IL-12 is typically not involved in the induction or regulation of T_{reg} cells. IL-12 does inhibit anergy induction, promotes the development of strong proliferative T cell response, and effector T cell function. IL-12 may act as a type of third signal, in addition to signals mediated by the TCR and costimulatory molecule-mediated signals, and reverse antigen-induced tolerance and expand antigen-specific T cells (28–30). One could speculate that the combination of IL-12 and GM-CSF tipped the balance, at least temporarily, in favor of T effector cells resulting in a high percentage of immunologic responses.

Important questions for future idiotype-vaccination trials revolve around three issues: selection of the appropriate patient

population, normalization of DC function, and overcoming the immunosuppressive activity of the various types of regulatory T cells. The myeloma patients most in need of novel strategies are those with a poor clinical outcome with high-dose chemotherapy combined with stem cell transplantation or application of novel drugs. These patients can be easily identified by gene expression profiling of purified plasma cells and exhibit a proliferation signature or translocations involving the FGFR3, c-MAF, or MAF-B loci (31). These patients may benefit from immunologic therapy after stem cell transplantation, when a state of minimal residual disease has been achieved, to eradicate residual chemorefractory myeloma cells. The preparative regimen could include drugs like fludarabine and cyclophosphamide, which eliminate T_{reg} cells (32, 33). However, patients are profoundly immunosuppressed post-peripheral blood stem cell transplantation, and it takes at least 3 months for the number of T cells to return to normal. The CD4⁺/8⁺ T-cell ratio remains inverted much longer and a severely abnormal T cell repertoire persists for at least 1 year (34, 35). A requirement would therefore be to vaccinate pre-transplantation, followed by collection and cryopreservation of idiotype-specific T cells, which could be returned prior to peripheral blood stem cell transplantation, when a lymphodepleted environment exists, followed by idiotype-booster vaccination to preferentially expand idiotype-specific T cells.

A different approach would be to target patients really early in their disease course with monoclonal gammopathy of underdetermined significance or smoldering myeloma. One would have to treat a very large number of patients and have long-term follow-up to establish clinical benefit. This disadvantage can perhaps be overcome by application of the newly developed serum-free light chain assay (36). Elevation of serum "freelites" in monoclonal gammopathy of underdetermined significance is associated with increased risk of disease progression. Reductions in serum freelites can rapidly indicate a response to vaccination and reflect the elimination of a more primitive monoclonal plasma cell population, which has lost the ability to secrete the complete IgG molecule. Novel vaccination strategies would have to take into account methods to restore normal DC function in patients with myeloma. Neutralizing antibodies to IL-6, IL-10, and transforming growth factor β or inhibition of p38 mitogen activated protein kinase can partially abrogate the detrimental effects of these cytokines on DCs (18, 37). Anti-IL-6 antibody may also restore the normal differentiation process of CD34⁺ cells to DCs. T_{reg} could be eliminated or reduced in frequency by anti-IL-25 antibody linked to diphtheria toxin. Antibodies to CTLA-4, glucocorticoid-induced tumor necrosis factor receptor-related gene, or CCR4 chemokine receptor could interfere with the induction and homing of T_{reg} cells (38). It is clear that we have entered a new era of vaccinology in which we have a better understanding of the immunoregulatory mechanisms that shape the type of immune response elicited by a vaccine. It is hoped that these insights will result in a new armamentarium for manipulating the immune system to enhance the activity of tumor vaccines.

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