

Idiotypic Immunity (Natural and Vaccine-induced) in Early Stage Multiple Myeloma

Anders Österborg, Lotta Henriksson and Håkan Mellstedt

From the Department of Oncology (Radiumhemmet) and Cancer Center Karolinska (CCK) (A. Österborg, L. Henriksson, H. Mellstedt) and the Department of Hematology (A. Österborg), Karolinska Hospital, Stockholm, Sweden

Correspondence to: Dr A. Österborg, Associate Professor, Department of Hematology, Karolinska Hospital, SE-171 76 Stockholm, Sweden. Tel: + 46 8 517 73385. Fax: + 46 8 31 8327. E-mail: aob@rah.ks.se

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Idiotypic structures expressed on the myeloma immunoglobulin (Ig) protein (M-component) might be regarded as tumor-specific antigens. Naturally occurring, idiotype-specific type I T-cell immunity has been observed preferentially in patients with early stage myeloma. The idiotypic structures on the clonal myeloma B-cells (B lymphocytes and plasma cells) may serve as targets for active immunization. Vaccination using the autologous monoclonal Ig as a vaccine has conferred resistance to tumor cell challenge in murine myeloma. The autologous myeloma Ig protein was used for immunization in patients with progressive stage I and early stage II multiple myeloma. When the idiotype (emulsified in aluminium phosphate) was used alone for immunization, a weak and transient idiotype-specific T-cell response was observed with no clinical effects. In our second series, the idiotype (in alum) was combined with GM-CSF. In all five patients, a specific T-cell response was induced consisting preferentially of MHC class I restricted (CD8+) T-cells. Ig-specific CD4+ T-cells were also induced. A clinical response (> 50% reduction of the M-component concentration) was observed in one patient. These results indicate that idiotype-specific T-cell immunity may be induced or enhanced by idiotype Ig vaccination in patients with early stage multiple myeloma, in which the tumor load is relatively low and the immune system is functionally less compromised than in patients with chemotherapy-treated, advanced stages of the disease. The use of GM-CSF seems to be mandatory for the frequency and magnitude of the induced T-cell response. The optimal route, schedule and cytokine combination for idiotype immunization remains to be established.

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Tumor cells express on the cell surface tumor-associated protein antigen molecules as well as peptides of the protein presented in the groove of MHC complexes. Although the vast majority of antigens are shared self antigens, operationally these structures can be regarded as tumor-specific antigens and used as targets for immunotherapy.

The tumor clone in multiple myeloma consists of plasma cells and clonogenic B lymphocytoid precursors. Plasma cells express usually only MHC class I molecules on their surface while B lymphocytes exhibit both MHC class I and class II antigens. In myeloma, clone-specific idiotypic immunoglobulin structures are expressed on the surface membrane of the malignant tumor cells and these structures might be an antigen for targeting (1). Idiotypic immunoglobulin molecules are unique antigens, normally involved in immune regulation. The surface-localized, tumor-derived idiotypes may elicit a humoral and cellular anti-idiotypic response (2, 3) that may control the growth of the myeloma B-cell clone. Active immunization using the autologous monoclonal Ig as a vaccine may confer resistance to tumor cell challenge in murine myeloma and B-cell lymphomas (4–6).

Thus, idiotype immunization could be a feasible strategy, especially in patients with early stage myeloma, in which the tumor load is low and the immune system is functionally less compromised than in advanced stages of the disease.

NATURAL IDIOTYPE IMMUNITY IN EARLY STAGE MULTIPLE MYELOMA

The presence of idiotype-specific blood T-cells in patients with multiple myeloma or MGUS has been studied by activating T-cells with the autologous myeloma-derived idiotypic immunoglobulin. Specific activation has been analysed by DNA synthesis (³H thymidine incorporation) and/or cytokine secretion (IFN- γ , IL-2, IL-4) (7). The number of specific cytokine-secreting cells can be enumerated in an enzyme-linked immunospot (ELISPOT) assay (8). Naturally occurring idiotype-specific T-cells were frequently detected in patients with indolent myeloma or MGUS (25). An idiotype-specific type I T-cell response (characterized by γ -interferon and IL-2 secretion), predominated in MGUS and early stage myeloma patients,

whereas a type II response pattern (IL-4 production) was preferentially seen in patients with a high tumor load (9). Idiotype reactivity was confined to both CD4+ and CD8+ T-cell subsets as well as to MHC class I- and II-restricted T-cells (10). The median total number of idiotype-specific blood T-cells in patients was calculated to be about $20/10^5$ PBMC, corresponding roughly to 1/5000 mononuclear cells (10). The fine specificity of idiotype-reactive T-cells was mapped using peptides corresponding to the complete CDR1-3 regions of the heavy and light chains as well as to MHC restricted sequences within the CDR2 and 3 heavy chain regions. T-cells specifically reacting with peptides corresponding to each of the three CDRs of the heavy chain variable part of the idiotype were found (11). Peptides corresponding to the CDRs of the variable part of the light chain of the monoclonal immunoglobulin or unrelated immunoglobulin fragments did not elicit a detectable idiotype-specific immune response (11–13). Taken together, these studies provide support for the existence of MHC restricted idiotype-specific T-cells which may target immunogenic idiotypic CDR peptides in monoclonal gammopathies. Such T-cells might be part of a naturally occurring, anti-tumor-specific immune response.

In addition, a humoral immunity (anti-idiotypic antibodies) has also been detected, especially in patients with MGUS and multiple myeloma stage I (14). Such antibodies are most likely to complex-bind *in vivo* to the tremendous excess of free circulating monoclonal immunoglobulin and may not be of importance for an immune defence against the tumor.

Moreover, clonal expansions of CD4+ as well as CD8+ T-cells have been noted (15–17). CD8+ clonal T-cell populations were more frequently seen in patients with a low tumor burden than in those with advanced disease (18). The relevance of such clonal T-cells with regard to recognition of malignant B-cells is not known. In one patient, two large expansions within the CD8+ subsets displayed no reactivity against the idiotype. Idiotype recognition was confined to a small non-expanded T-cell population but with a monoclonal restricted TCR usage (TCRBV22) (18).

In a study by Brown et al., the presence of clonally expanded T-cells correlated with the presence of idiotype-reactive T-cells. A dominant TCRBV rearrangement (CD4 or CD8) was significantly more frequent in patients with progressive disease, but the presence of a dominant rearrangement conferred a survival advantage during the progressive phase of the myeloma disease (19). The presence of dominant T-cell clone(s) may be a good prognostic factor in multiple myeloma patients.

IDIOTYPE IMMUNIZATION IN PATIENTS WITH EARLY STAGE MULTIPLE MYELOMA

As indicated above, tumor-derived idiotypic immunoglobulin may elicit a humoral and cellular anti-idiotypic re-

sponse, which conferred resistance to tumor cell challenge in animal models. A rewarding therapeutic approach might thus be to immunize myeloma patients with the autologous idiotype. Patients with early stage myeloma may be particularly suitable for vaccination because, as indicated above, a natural pre-existing idiotype-specific immunity may be present which may be enhanced/amplified by the vaccine. In addition, these patients have a relatively low tumor burden and may be less immunocompromised than those with advanced disease who are heavily pretreated with chemotherapy.

A relatively simple procedure for vaccination of patients, which can easily be adopted in a large number of patients, is to use the autologous idiotypic immunoglobulin isolated from serum and conjugated to/emulsified in a strong immune-enhancing adjuvant. In the first series, five patients with stage I multiple myeloma were immunized with the autologous serum M-component alone precipitated in alum. An idiotype-specific T-cell immunity was induced in two of the patients but the T-cell response was of a modest magnitude and of short duration (20). No clinical effects were seen. The addition of adjuvant cytokines may improve the results. GM-CSF may be a cytokine of major importance for induction of immunity in cancer vaccination. Exogenous antigens are taken up, processed and presented in the context of MHC molecules on the cell surface of antigen presenting cells (APC). Both class I and II presentation pathways might be used (21). The most important APC are dendritic cells, monocytes and B-cells. Dendritic cells and monocytes must be activated to be able to present antigens. GM-CSF activates these cells (22–24). The primary cellular immune response might also be amplified by the addition of GM-CSF because GM-CSF may trigger naive T-cells as well as the humoral response, which may also be enhanced by GM-CSF – as noted in patients immunized with hepatitis B and influenza antigens (23, T Jones, personal communication). We have shown in colorectal carcinoma patients that GM-CSF was almost a mandatory addition to the auto-antigen CEA for the induction of a humoral and T-cell response. Moreover, the specific T-cell response was type I MHC class II- and class I-restricted (25). Furthermore, immunization with GM-CSF-transfected tumor cells induced protective as well as therapeutic immunity in animals (26).

Because GM-CSF was found to be a potent stimulator for the induction of a specific anti-tumor immunity in several animal tumor models (26, 27), we decided to evaluate the effect of this cytokine in our second series (28). Five patients with indolent multiple myeloma were immunized intracutaneously with the autologous idiotypic immunoglobulin (precipitated in alum) at day 1 together with 75 μ g/day of GM-CSF s.c. at days 1–4 at the same site. The immunization procedure was repeated at weeks 2,

4, 6, 8 and 14. An idiotype-specific T-cell immunity was induced in all five patients. In four of five patients, predominantly CD8+ MHC class I-restricted idiotype-specific T-cells were noted but CD4+ MHC II-restricted T-cells were also induced. Epitope mapping revealed a reactivity against peptides corresponding to the CDR 1, 2 and 3 regions of the heavy chains but not to CDR peptides from idiotypic proteins of other (control) myeloma patients. The magnitude of the T-cell response was higher and the duration of the specific T-cell immunity was longer compared with the first series (where GM-CSF was not given). In one of these patients the M-component concentration was reduced by > 50% (28).

Another cytokine that may also be of interest in the development of a vaccine strategy is IL-12. IL-12 is mainly produced by APC (DC, monocytes, B-cells) and is of importance for the generation of Th1 cells and for the augmentation of CTL activity (29, 30). IL-12 is a growth factor for preactivated T-cells and enhances cytotoxic activity of CD8+ T-cells (31, 32). IL-12 has been used to boost a protective as well as a therapeutic immunity in animal tumor models (33, 34). IL-12 was also effective in inducing a protective immunity against poorly immunogenic animal tumors (33). In addition to activating T lymphocytes with a predominant systemic activation of Th1 more than Th2 cells, IL-12 induced complement-fixing anti-tumor antibodies contributing to the tumor cell destruction (35). Moreover, IL-12 administration prolonged survival in tumor-bearing mice by activating pre-existing Th1 cells (36).

These results indicate that both IL-12 and GM-CSF might be important cytokines for the enhancement of a specific immunity against tumor-associated antigens including idiotypes.

A phase I–II trial has been initiated to analyse whether IL-12 together with the autologous idiotype or whether the combination of IL-12 and GM-CSF together with the autologous idiotype may induce a specific T-cell response in patients with early stage myeloma. The induction immunization schedule is identical to that of our latest trial. To maintain a long-lasting immunity for low avidity antigens (such as idiotypes), repeated immunization should continue for a long period of time (37). In this study, vaccination will be accomplished for 2 years. Preliminary data from this ongoing trial indicate that there may be a large interpatient variability with regard to the time kinetics of the induced idiotype-specific T-cell response (38). In some patients, a rapid increment in the magnitude of the idiotype-specific T-cell response is seen, while in others the specific response was not detectable until the end of the immunization period, as recently shown also by Reichardt et al. (39). Whether repeated immunizations may lead to maintenance and augmentation of a specific T-cell response or, alternatively, induction of T-cell anergy, remains to be shown.

CONCLUSIONS

Taken together, these results indicate that idiotype-specific immunity can be induced or enhanced in patients at an early stage of the disease. Several issues remain to be addressed in future clinical studies on idiotype vaccination in patients with multiple myeloma. The potential dormancy-inducing effect of the excess of free circulating monoclonal Ig, which may lead to induction of tolerance or T-cell anergy in relation to the idiotype, needs to be taken into account (40). Idiotype-specific T-cells may also be clonally deleted, resulting in tumor escape from immunosurveillance (41). This potential obstacle may speak in favor of vaccination in a minimal residual disease situation, where circulating idiotypic Ig is absent. On the other hand, the immunosuppression induced by high-dose chemotherapy may favor vaccination during the very early course of the disease. The excess of circulating monoclonal Ig underscores the importance of focusing on the induction of a T-cell response rather than antibodies, which may be simply blocked by the M-protein. It remains also to be formally demonstrated that myeloma tumor cells express idiotypes in an MHC–peptide complex form on the cell surface to be targeted by T-cells. Another important question is whether idiotype-specific T-cells provide T- to B-cell help and drive the B-cell clone (42). In an animal model, T-cells were described that specifically facilitated terminal tumor B-cell differentiation, with a shift from a B-cell lymphoma to a plasmacytoma (43). Most importantly for further study in extended trials in human myeloma is whether idiotype vaccination has clinical effects.

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REFERENCES

1. Stevenson GT, Stevenson FK. Antibody to molecularly-defined antigen confined to a tumor cell surface. *Nature* 1975; 254: 714–6.
2. Hoover RG, Kornbluth J. Immunoregulation of murine and human myeloma. *Hematol Oncol Clin North Am* 1992; 6: 407–24.
3. Yi Q, Österborg A. Idiotype-specific T-cells in multiple myeloma: targets for an immunotherapeutic intervention? *Med Oncol* 1996; 13: 1–7.
4. Lynch RG, Graff RJ, Sirisinha S, et al. Myeloma proteins as tumor-specific transplantation antigens. *Proc Natl Acad Sci USA* 1972; 69: 1540–4.
5. Daley MJ, Gebel HM, Lynch RG. Idiotype specific transplantation resistance to MOPC-315: abrogation by post-immunization thymectomy. *J Immunol* 1978; 120: 1620–4.
6. George AT, Folkard SG, Hamblin TJ. Idiotypic vaccination as a treatment for a B-cell lymphoma. *J Immunol* 1988; 141: 2168–74.

7. Österborg A, Yi Q, Bergenbrant S, et al. Idiotype-specific T-cells in multiple myeloma stage I: an evaluation by four different functional tests. *Br J Haematol* 1995; 89: 110–6.
8. Yi Q, Bergenbrant S, Österborg A, et al. T-cell stimulation induced by idiotypes on monoclonal immunoglobulins in patients with monoclonal gammopathies. *Scand J Immunol* 1993; 38: 529–34.
9. Yi Q, Österborg A, Bergenbrant S, et al. Idiotype-reactive T-cell subsets and tumor load in monoclonal gammopathies. *Blood* 1995; 86: 3043–9.
10. Yi Q, Eriksson I, He W, et al. Idiotype-specific T lymphocytes in monoclonal gammopathies. Evidence for the presence of CD4+ and CD8+ subsets. *Br J Haematol* 1997; 96: 338–45.
11. Fagerberg J, Yi Q, Gigliotti D, et al. T-cell epitope mapping of the idiotype monoclonal IgG heavy and light chains in multiple myeloma. *Int J Cancer* 1999; 80: 671–80.
12. Wen Y-J, Ling M, Lim SH, et al. Immunogenicity and cross-reactivity with idiotype IgA of VH CDR3 peptide in multiple myeloma. *Br J Haematol* 1998; 100: 464–8.
13. Dabaghao S, Bergenbrant S, Anton, et al. Anti-idiotypic T-cell activation in multiple myeloma induced by M-component fragments presented by dendritic cells. *Br J Haematol* 1998; 100: 647–54.
14. Bergenbrant S, Österborg A, Holm G, et al. Anti-idiotypic antibodies in patients with monoclonal gammopathies: relation to the tumour load. *Br J Haematol* 1991; 78: 66–70.
15. Janson CH, Grunewald J, Österborg A, et al. Predominant T-cell receptor V gene usage in patients with abnormal clones of B-cells. *Blood* 1991; 77: 1776–80.
16. Moss P, Gillespie G, Frodsham P. Clonal populations of CD4+ and CD8+ T-cells in patients with multiple myeloma and paraproteinemia. *Blood* 1996; 87: 3297–306.
17. Halapi E. Oligoclonal T cells in human cancer. *Med Oncol* 1998; 15: 203–11.
18. Halapi E, Werner Å, Wahlström J, et al. T-cell repertoire in patients with multiple myeloma and monoclonal gammopathy of undetermined significance – clonal CD8+ T-cell expansions are found preferentially in patients with a low tumor burden. *Eur J Immunol* 1997; 27: 2245–52.
19. Brown RD, Yuen E, Nelson M, et al. The prognostic significance of T-cell receptor beta gene rearrangements and idiotype-reactive T-cells in multiple myeloma. *Leukemia* 1997; 11: 1312–7.
20. Bergenbrant S, Yi Q, Österborg A, et al. Modulation of anti-idiotypic immune response by immunization with the autologous M-component protein in multiple myeloma patients. *Br J Haematol* 1996; 92: 840–6.
21. Rock KL. A new foreign policy: MHC class I molecules monitor the outside world. *Immunol Today* 1996; 17: 131–7.
22. Cauz C, Dezutter-Dambuyant C, Schmitt D, et al. GM-CSF and TNF- α cooperate in the generation of dendritic Langerhans cells. *Nature* 1992; 360: 258–61.
23. Fischer HG, Frosch S, Reske K, et al. Granulocyte-macrophage colony-stimulating factor activates macrophages derived from bone marrow cultures to synthesis of MHC class II molecules and to augmented antigen presentation function. *J Immunol* 1988; 141: 3882–8.
24. Morrissey PJ, Bressler L, Park LS, et al. Granulocyte-macrophage colony-stimulating factor augments the primary antibody response by enhancing the function of antigen-presenting cells. *J Immunol* 1987; 139: 1113–9.
25. Samanci A, Yi Q, Fagerberg J, et al. Pharmacological administration of granulocyte/macrophage-colony stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. *Cancer Immunol Immunother* 1998; 47: 131–42.
26. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific and long-lasting antitumor immunity. *Proc Natl Acad Sci USA* 1993; 90: 3539–43.
27. Kwak LW, Young HA, Pennington RW, et al. Vaccination with syngeneic lymphoma-derived immunoglobulin idiotype combined with granulocyte/macrophage colony-stimulating factor primes mice for a protective T-cell response. *Proc Natl Acad Sci USA* 1996; 93: 10972–7.
28. Österborg A, Yi Q, Henriksson L, et al. Idiotype immunization combined with GM-CSF in myeloma patients induced MHC-restricted, CD8 and CD4 specific T-cell responses. *Blood* 1998; 91: 2459–66.
29. Trinchieri G. Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type I and cytotoxic lymphocytes. *Blood* 1994; 84: 4008–27.
30. Mehrotra PT, Wu D, Crim JA, et al. Effects of IL-12 on the generation of cytotoxic activity in human CD8+ T lymphocytes. *J Immunol* 1993; 151: 2444–52.
31. Wolf SF, Temple PA, Kobayaski M, et al. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J Immunol* 1991; 146: 3074–81.
32. Gubler U, Chua AO, Schoenhaut DS, et al. Coexpression of two distinct genes is required to generate bioactive cytotoxic lymphocyte maturation factor. *Proc Natl Acad Sci USA* 1991; 88: 4143–7.
33. Vagliani M, Rodolfo M, Cavallo F, et al. Interleukin 12 potentiates the curative effect of a vaccine based on interleukin-2-transduced tumor cells. *Cancer Res* 1996; 56: 467–70.
34. Irvine KR, Rao JB, Rosenberg SA, et al. Cytokine enhancement of DNA immunization leads to effective treatment of established pulmonary metastases. *J Immunol* 1996; 156: 238–45.
35. Rodolfo M, Zilocchi C, Melani C, et al. Immunotherapy of experimental metastases by vaccination with interleukin gene-transduced adenocarcinoma cells sharing tumor-associated antigens. Comparison between IL-12 and IL-2 gene-transduced tumor cell vaccines. *J Immunol* 1996; 157: 5536–42.
36. Tsung K, Meko JB, Peplinski GR, et al. IL-12 induces T helper 1-directed antitumor response. *J Immunol* 1997; 158: 3359–65.
37. Fairchild PJ, Wraith DC. Lowering the tone: mechanisms of immunodominance among epitopes with low affinity for MHC. *Immunol Today* 1996; 17: 80–5.
38. Österborg A, Henriksson L, Yi Q, et al. Idiotype vaccination in early stage myeloma patients. International Workshop of Multiple Myeloma, Stockholm, 1–5 September 1999 [abstr].
39. Reichardt VL, Okada CY, Liso A, et al. Idiotype vaccination using dendritic cells after autologous peripheral blood stem cell transplantation for multiple myeloma. A feasibility study. *Blood* 1999; 93: 2411–9.
40. Bogen B. Peripheral T-cell tolerance as a tumor escape mechanism: deletion of CD4+ T-cells specific for a monoclonal immunoglobulin idiotype secreted by a plasmacytoma. *Eur J Immunol* 1996; 26: 2671–9.
41. Lauritzsen GF, Horgaard PO, Schenk K, et al. Clonal deletion of thymocytes as a tumor escape mechanism. *Int J Cancer* 1998; 78: 216–22.
42. Clark EA, Ledbetter JA. How B and T-cells talk to each other. *Nature* 1994; 367: 425–8.
43. Hilbert DM, Shen MY, Rapp UR. T-cells induce terminal differentiation of transformed B-cells to mature plasma cell tumors. *Proc Natl Acad Sci USA* 1995; 92: 649–53.