Tumor idyotipe as target antigen for multiple myeloma

[haematologica reports]
2005;1(8):57-59

CURTI A

Institute of Hematology and Medical Oncology L & A Seragnoli, University of Bologna, Italy

Early during development, pre-B cells become committed to the expression of a heavy and light chain Ig variable region. The heavy chain derives from the recombination of a variable (V) with a diversity (D) and a joining (J) region genes with a constant region (C). The V-D-J joints occur with a variable number of nucleotide insertions or deletions resulting in a unique sequence which creates the third hypervariable region (CDR III) and contributes to the antigen binding site. These antigenic regions (idiotype; Id) are characteristic for any given Ig producing tumor (i.e. multiple myeloma, MM; non-Hodgkin lymphoma; NHL) and can be recognized by an immune response consisting of anti-Id antibodies and/or by Id reactive T-cells. The tumor derived Id is a self protein which is in most circumstances poorly immunogenic. However, it can be made immunogenic if it is coupled to a carrier protein and administered in an immunological adjuvant formulation. In a murine lymphoma model (38C-13) the Id was found to be highly immunogenic when conjugated to the protein keyhole limpet hemocyanin (KLH) and a potent polyclonal anti-Id antibody response could be generated to protect mice from subsequent challenge with letal numbers of lymphoma cells (reviewed in 1). Resistance to the MOPC-315 plasmacytoma cell line was demonstrated following Id immunization. In this model, Id-reactive $CD4^+$ lymphocytes were generated upon presentation of Id peptides on MHC class II molecules of antigen-presenting cells to T-cells.[1] Adoptive therapy with these $CD4^+$ T-cells inhibited the growth of the Ig secreting myeloma cells. Cytotoxic $CD8^+$ lymphocytes (CTL), specific for a class I presented peptide fragments, have been more elusive, but have been described in one model system.[1] At the University of Stanford, a series of patients with B-cell NHL have been vaccinated with their autologous tumor Id conjugated to the carrier protein KLH and administered with an immunological adjuvant.[2] The majority of the patients mounted an Id-restricted antibody response and some of them showed tumor regression. A T-cell proliferative response was rarely observed. Similar to the B-cell NHL, the Id of myeloma tumor cells is a tumor specific antigen. However, there are important differences between NHL cancer cells and MM plasma cells. In the case of NHL, the cells are characterized by high surface expression and little antibody secretion whereas myeloma cells have very low levels of cell surface Ig with high levels of antibody secretion. Thus, it is unlikely that the generation of an antibody based anti-Id immune response will be beneficial for MM patients. In fact, anti-Id antibodies may be blocked from reaching the tumor cells by the high levels of circulating Id. Moreover, despite the existence of a pre-plasma cell stem cell compartment in MM with a higher expression of surface Ig it is possible that tumor cells would not express enough levels of the target protein for the antibodies to be effective. Conversely, an Id specific T-cell response would not need to bind to cell surface Ig to be active. T-cells do not recognize intact protein, but are specific for processed peptide fragments of the Id expressed on class I or II molecules. The advantage of a cytotoxic T-cell response is that it would not be blocked by free circulating paraprotein and would not depend on the expression of the native protein on the surface of tumor cells. Moreover, B-cells, including putative myeloma stem cells, are known to process and present peptides of the Ig on their membrane associated with class I and II molecules. Therefore, optimal strategies for Id vaccination will likely require the induction of a T-cell-mediated immune response which is best achieved by the use of professional antigen presenting cells (APC). Interestingly, it has recently been described[3] the immunization of a matched related donor of allogeneic BM with the myeloma derived Id (conjugated with KLH) isolated pre-transplant from the patients. After transplantation, a $CD4^+$ T-cell line was established from the recipient PBL and found to be of donor origin and proliferate

specifically in response to the myeloma Id. Thus, this experience demonstrates the principle of transfer of donor immunity with the advantage of immunizing a tumor naive donor who may be more likely able to generate an immune response against the tumor Ig.

Dendritic cells

Among professional APC, dendritic cells (DC) are specialized in capturing and processing antigens into peptide fragments that bind to MHC molecules.[4] DC are the most potent stimulators of T-cell responses and they are unique in that they stimulate not only sensitized but also naive T-cells. Thus, DC appear critical (*nature's adjuvant*) for the induction of T-cell-mediated immune responses. Recent evidence supports the role of DC in tumor immunity. Murine DC pulsed with tumor-associated protein or peptides or transduced with TAA genes, have been shown capable of inducing a specific antitumor response *in vitro* and *in vivo*.[4] In humans, DC pulsed with tumor-Id caused T-cell and humoral tumor-specific immune responses and regression of chemotherapy-resistant malignant lymphoma.[5,6] Noteworthy, these studies showed the efficacy of DC-based vaccination even in case of established tumors. It is, in fact, long-standing experience in tumor immunology that due to tolerance induction it is most difficult to immunize tumor bearing hosts. However, DC are known to be capable of breaking tolerance. Preliminary data on DC vaccination of MM patients indicate the possibility of stimulating, in some cases, an anti-Id T-cell response. However, the immune response was not associated with any clinical benefit perhaps due to the high tumor burden of patients enrolled in the clinical trial and the use of circulating DC which appear to be less immunogenic than ex-vivo generated DC (see below).[7] DC can be generated *ex-vivo* from $CD34^+$ progenitor cells[4,8,9] and more mature precursors in presence of appropriate cytokines. We recently showed that peripheral blood (PB) $CD14^+$ monocytes (Mo) from MM patients can be induced to differentiate into fully functional, mature, $CD83^+$ DC.[10] Following immunomagnetic adsorption of $CD14^+$ cells, the Mo were stimulated in serum-repleted cultures by a combination of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4), with sequential use of tumor necrosis factor-α (TNF-α) as a maturation factor. The resulting $CD83^+$ DC-enriched cell population was highly efficient in priming allogeneic and autologous T lymphocytes in response to the patient-specific tumor Id. Whereas Mo-DC are efficient in tumor-Ag presentation, we have also shown that circulating DC isolated from MM patients have an impaired capacity of presenting the patient-specific Id to T cells,[11] and $CD34^+$ cells, another tested source of DC,[12] have a limited proliferative potential and provide a lower a yield.[10]

Anti-Id vaccination with dendritic cells: results of Bologna clinical trial

Thirteen multiple myeloma (MM) patients were treated with two courses of high-dose chemotherapy with peripheral blood stem cell support and then entered in a clinical study of anti- Id. vaccination with dendritic cells (DC). DC were generated from positively selected circulating monocytes according to good manufacturing practice guidelines, in FCS-free medium in cell culture bags, in presence of GM-CSF plus IL-4 followed by either TNF-α or a cocktail of IL-1-β, IL-6, TNF-α and prostaglandin-E2. $CD14^+$ monocytes were enriched from $16.1\pm5.7\%$ to $95.5\pm3.2\%$ (recovery 67.9 ± 15 %, viability >97%). After cell culture, phenotypic analysis showed that $89.6\pm6.6\%$ of the cells were DC: we obtained $2.89\pm1\times10^8$ DC/leukapheresis which represented $24.5\pm9\%$ of the initial number of $CD14^+$ cells. Notably, the cytokine cocktail induced a significantly higher percentage and yield (31 ± 10.9 of initial $CD14^+$ cells) of DC than TNF-α alone, secretion of larger amounts of IL-12, potent stimulatory activity on allogeneic and autologous T cells. Storage in liquid nitrogen did not modify the phenotype or functional characteristics of pre-loaded DC. The recovery of thawed, viable DC, was $78\pm10\%$. Ten patients in partial remission after autologous stem cell transplantation received a series of by-monthly immunizations consisting of three subcutaneous and two intravenous injections of Id-keyhole limpet hemocyanin (KLH)-pulsed DC (5×-, 10×-, 50×10^6 cells and 10×-, 50×10^7 cells, respectively). The patient-specific Id was used as whole protein in 4 patients whereas 6 additional patients had their DC charged with Id (VDJ)-derived HLA class I restricted peptides. The administration of Id-pulsed DC was well tolerated with no clinically significant side effects. So far, 6 patients have been fully evaluated for their immunological response to DC vaccination. Six of 6 patients developed a humoral and T-cell proliferative response to KLH. Moreover, 5/6 showed circulating IFN-γ-secreting T cells by Elispot. None of the patients mounted a B-cell response to Id whereas 6/6 developed a Id-specific T-cell proliferative response and 4/6 IFN-g-secreting T cells. Delayed-type hypersensitivity (DTH) tests showed 6/8 and 2/8 patients responsive to KLH and tumor Id, respectively. Ten out of thirteen patients have completed vaccination schedule. With a median follow up of 20 months, 4/10 patients have stable disease, 2 patients are in CR, 1 patient obtained clinical response and 3 patients progressed. In summary, positive selection of circulating $CD14^+$ monocytes allows the generation of mature and functional DC suitable for clinical trials and cryopreservation does not affect the phenotype and function of pre-loaded DC. Moreover, injections of cryopreserved DC pulsed with tumor Id or Id-derived peptides are safe and induce T-cell tumor-specific responses.

References

1. Bocchia M, Bronte V, Colombo MP, De Vincentiis A, Di Nicola M, Forni G, et al. Antitumor vaccination: where we stand. Haematologica, 2000;85:1172-1206.
2. Hsu FJ, Caspar CB, Czerwinski D. Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma: long-term results of a clinical trial. Blood 1997;89: 129-135.
3. Kwak LW, Taub DD, Duffey PL, et al. Transfer of myeloma idiotype-specific immunity from an actively immunised marrow donor. Lancet 1995;345: 1016-20.
4. Bancherau J, Steinman R.M. Dendritic cells and the control of immunity. Nature 1998;392, 245-52.
5. Hsu FJ, Benike C, Fagnoni F, Liles TM, Czerwinski D, Taidi B, et al. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. Nature Medicine 1996;2, 52-58.
6. Timmerman JM, Czerwinski DK, Davis TA, Hsu FJ, Benike C, Hao ZM, et al. Idiotype pulsed dendritic cell vaccination for B-cell lymphoma: clinical and immune responses in 35 patients. Blood 2002; 99, 1517-26.
7. Reichardt VL, Okada CY, Liso A, Benike CJ, Stockerl-Goldstein KE, Engleman EG 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.
8. Ratta M, Rondelli D, Fortuna A, Curti A, Fogli M, Fagnoni F, Martinelli G, Terragna C, Tura S, Lemoli RM. Generation and functional characterization of human dendritic cells derived from $CD34^+$ cells mobilized into peripheral blood: comparison with bone marrow $CD34^+$ cells. Br J Haematol 1998;101: 756-65.
9. Curti A, Fogli M, Ratta M, Tura S, Lemoli RM. Stem cell factor and FLT3-Ligand are strictly required to sustain the long-term expansion of primitive CD34+DR- dendritic cell precursors. Journal of Immunology 2001;166: 848-54.
10. Ratta M, Curti A, Fogli M, Pantucci M, Viscomi G, Tazzari PL, et al. Efficient presentation of tumor idiotype to autologous T-cells by CD83+ dendritic cells derived from highly purified circulating CD14+ monocytes in multiple myeloma patients. Exp Hematol 2000;28, 931-40.
11. Ratta M, Fagnoni F, Curti A, Vescovini R, Sansoni P, Oliviero B et al. Dendritic cells are functionally defective in multiple myeloma: The role of interleukin-6. Blood 2002;100, 230-7.
12. Titzer S, Christensen O, Manzke O, Tesch H, Wolf J, Emmerich B, et al. Vaccination of multiple myeloma patients with idiotype-pulsed dendritic cells: immunological and clinical aspects. Br J Haematol 2000;108, 805-16.