Ganglioside–based vaccines and anti–idiotype antibodies for active immunotherapy against cancer

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This review shall present an update in anticancer ganglioside-based immunotherapies, with particular emphasis on molecular vaccines and anti-idiotype monoclonal antibodies produced by the Center of Molecular Immunology (Havana, Cuba). The project comprises vaccines of *N*-acetyl- or *N*-glycolylneuraminic acid GM3 ganglioside incorporated into very small proteoliposomes and anti-idiotype antibodies to glycolylated gangliosides. Development of these vaccine preparations from preclinical models of melanoma, breast and lung cancer to human investigation is summarized. A brief discussion on the progress and limitations of present-day clinical trials and future prospects is also included.

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Immunotherapy is a promising field in cancer research and numerous approaches are being investigated to develop effective cancer vaccines. Classically, the aim of active specific immunotherapy (ASI) is to teach the host immune system to recognize antigens expressed in the tumor and therefore be able to destroy abnormal cells leaving the normal tissue intact.

For many years, concepts for development of successful immunotherapy of cancer revolved around the induction of immune responses against tumor 'neoantigens'. Unfortunately, this type of antigen was only represented in human tumors by individual changes in amino acids in common proteins. The generation of tissue-specific autoimmune responses represents an approach to cancer immunotherapy that is gaining momentum [1,2]. However, in classical autoimmune diseases, only normal cells and tissues are the targets involved. The main obstacle to achieving an effective immunotherapy of cancer is the genetic instability of tumors and the consequent adaptation of transformed cells to survive in a hostile immunological environment [3].

Certainly, it is unlikely that successful cancer vaccines will, in themselves, represent a total solution to the cancers against which they are directed until emerging technologies allow us to solve the question of tumor editing [4]. Meanwhile, it is more likely that they will form part of a multiple approach against residual disease. In such cases, some of the potential roles of cancer vaccines may be as adjuvant therapy – following surgery, radiation or chemotherapy – to reduce the tumor mass in cases of unresectable disease, or as a maintenance therapy to ensure an active immune response against future relapses [5,6].

The use of immunotherapeutic vaccines in high-risk cancer patients in combination with sensitive methods to detect minimal residual disease would be an attractive treatment modality. Recently, several reverse transcription-polymerase chain reaction (RT-PCR) techniques have proven to be useful for the detection of circulating tumor cells using different markers, including tyrosinase in melanoma [7], maspin in breast cancer [8] and telomerase in hematological malignancies [9] and in most solid cancers [10,11]. Recent reports suggest that circulating tumor cells are markers of a high relapse risk and shorter disease-free survival during follow-up and may be a useful tool for monitoring adjuvant treatment [12]. The next decade will indeed see an increasing number of attempts to harness cancer vaccines with techniques to monitor the status of each individual treatment.

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In this review, an update on anticancer ganglioside-based immunotherapies shall be presented, with particular emphasis on molecular vaccines and anti-idiotype antibodies produced by the Center of Molecular Immunology (Havana, Cuba). The project comprises vaccines of GM3 ganglioside containing *N*-acetylneuraminic acid (*N*AcGM3) or *N*-glycolylneuraminic acid (*N*GcGM3), incorporated into very small size proteoliposomes (VSSP) and anti-idiotype monoclonal antibodies to glycolated gangliosides.

Ganglioside antigens

Interest in gangliosides as potential targets for ASI of cancer began 27 years ago when Irie and Morton observed that patient's immunoglobulin (IgM) antibodies reactive against melanoma membrane antigens also reacted against ganglioside-rich neural tissues of fetal brain [13]. The next 15 years were relevant for the field of gangliosides and cancer due to three main facts:

- GM2 and GD2 were unequivocally identified as melanoma antigens [14,15]
- Major clinical responses were seen following treatment of melanoma patients with monoclonal antibodies against GM2, GD2 and GD3 [16–18]
- A correlation between the presence of anti-GM2 natural serum antibodies and survival in Stage III melanoma patients was reported [19]

Gangliosides, which are a family of structurally related molecules, have been considered attractive targets based on their higher abundance in tumors when compared with the corresponding normal tissues [20]. In particular, this relative abundance was highlighted for its association with specific antibodies, the number of molecules necessary for efficient complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC) and the mechanism for an antitumor response. Based on this, the ganglioside vaccine approach was considered to be potentially effective as a 'generic' cancer immunotherapy. Another advantage of this generic conception is to provide rationale for polyvalent vaccines [21].

However, the basis for the selection of an effective target for cancer vaccines could be a qualitative criterion rather than a quantitative one. The biological significance of the selected molecule, as well as its connection with tumor biology and dynamic, is indeed a pivotal issue. In our GM3 gangliosides-based ASI this qualitative perspective is considered. *N*AcGM3 have been implicated in a variety of biological events. In T-lymphocytes, *N*AcGM3 represents the main ganglioside constituent of the plasma membrane (72% of total lipid-bound sialic acid). Important molecules for T-cell function, such as CD4 coreceptors, have been associated with GM3-enriched plasma membrane microdomains. *In vitro* exogenous addition of *N*AcGM3 to human peripheral blood lymphocytes induced rapid endocytosis of cell-surface CD4 [22], interleukin (IL)-10 secretion and mRNA expression [23]. These and other examples of the effects of this ganglioside over a variety of immune cells, including the induction of specific suppressor T-cells, are the basis for the hypothesis that shedding of *N*AcGM3 by tumor cells is a successful mechanism to avoid destruction by immune effectors [24,25]. In addition, important evidence of the contribution of *N*AcGM3 to tumor invasiveness and formation have been obtained from *in vitro* [26] and *in vivo* [27] studies. Other authors have also described GM3-enriched microdomains in melanoma cells and postulated that these microclusters are involved in vital tumor cell functions, including adhesion, migration and cell signaling, by interaction of GM3 with s-Src and Rho transducer proteins [28,29]. These support further evaluation of *N*AcGM3 as a privileged target for ASI in cancer.

Whereas gangliosides containing *N*eu5Ac are normal components of the plasma membrane in humans, gangliosides bearing the *N*GcGM3 residue (*N*eu5Gc) are not expressed in normal cells. The lack of expression of this type of sialic acid in humans is due to inactivation by a deletion of the *CMP-Neu5Ac* hydroxylase gene, the enzyme responsible for *N*eu5Gc biosynthesis [30–32]. However, the presence of *N*GcGM3 residues has been reported in different human tumors, detected by polyclonal and monoclonal antibodies [33–37]. Of particular relevance is our report of increased amounts of *N*GcGM3 in human breast tumors by biochemical methods [38]. These immunochemical and analytical data have confirmed the presence of this molecule in human tumors [37], revitalizing the old dream of cancer immunotherapy related to the existence of real tumor-specific antigens. Noteworthy, recent data have shown that the majority of human anti-non-Gal antibodies (responsible for antibody hyperacute rejection) are specific for carbohydrate structures carrying terminally linked *N*eu5Gc. These antibodies were detected in 85% of healthy humans and are most likely involved in hyperacute rejection [39], meaning that anti-*N*GcGM3 antibodies could be particularly valuable for cancer treatment.

Anti-idiotype antibodies & molecular vaccines

An alternative approach to generate an effective immune response against gangliosides involves the use of an anti-idiotype monoclonal antibody (Ab2 mAb), bearing the 'internal image' of the antigen. Certain anti-idiotype antibodies that mimic tumor-associated antigens can be used as surrogate antigens for ASI. The use of Ab2 as a vaccine is a consequence of Jerne's idiotypic network theory, which postulated that the Ig idiotypic repertoire must mimic the universe of nonself and self antigens [40].

Our group have previously described an Ab2 monoclonal antibody to a murine Ab1 monoclonal antibody, designated as P3, which recognizes *N*euGc-containing gangliosides and antigens expressed in human breast tumors and melanoma [35,41]. This IgG_1 Ab2 monoclonal antibody, named 1E10, was capable of blocking the binding of the Ab1 monoclonal antibody (mAb) to *N*GcGM3.

In the case of molecular vaccines, hydrophobic incorporation into very small size proteoliposomes (VSSP technology) [42] allows the placement of gangliosides within potent Gram-negative innate immunity ligands, ensuring they remain structurally intact. Ganglioside incorporation into bacterial outer-membrane proteins leads to a dramatic reduction in the size of the VSSP complex which, contrary to other proteosome-based preparations, is basically nanoparticulated (average diameter of 6–8 nm). One important consequence of this is that VSSP preparations are quite transparent solutions that can be easily sterilized by filtration through 0.22-µm membrane filters. Studies on the immunogenicity of VSSP demonstrated that not only IgM but also high-level IgG antiganglioside antibodies can be induced in mice, chickens and monkeys.

Preclinical data

In recent years, our group have been analyzing the antitumor activity and the preclinical toxicology of 1E10 anti-idiotype monoclonal antibody and the molecular vaccines *N*AcGM3/VSSP and *N*GcGM3/VSSP using different animal models.

Acknowledging that the most important criterion for the selection of an Ab2 monoclonal antibody to be used in immunotherapy should be its biological effect, the 1E10 vaccine was evaluated in two syngeneic murine tumor models, the F3II mammary carcinoma (BALB/c mice) and B16 melanoma (C57BL/6 mice). Both cell lines are positive for the idiotype mAb P3, which specifically reacts with *N*-glycolyl-containing gangliosides. In BALB/c mice, vaccination with several intraperitoneal doses at 14-day intervals of 1E10 coupled to keyhole limpet hemocyanin in Freund's adjuvant, significantly reduced subcutaneous tumor growth of F3II carcinoma cells and the formation of spontaneous lung metastases. The effect of 1E10 as a biological response modifier on lung colonization was evaluated in C57BL/6 mice injected with B16 melanoma cells. Interestingly, intravenous administration of uncoupled 1E10 antibody, 10 to 14 days after inoculation of tumor cells, dramatically reduced the number of experimental metastases in comparison with lungs from mice treated with an irrelevant IgG [43]. The antimetastatic effect produced by 1E10 was also observed using 3LL-D122 lung carcinoma cells.

With respect to the ganglioside-based vaccines, the antitumor activity of *N*AcGM3/VSSP plus the immunological adjuvant Montanide ISA51™ (SEPPIC, Inc, Paris, France) was evaluated in the B16 melanoma model [44,45]. Vaccines were administered intramuscularly in the quadriceps at 14-day intervals and B16 cells were injected in the subcutis of the right flank of C57BL/6 mice. Significant suppression of tumor growth and prolongation of survival were seen by preimmunization with at least four doses of *N*AcGM3/VSSP vaccine in animals challenged with small subcutaneous tumor burdens (less than 10^4 live melanoma cells). Vaccination also reduced tumor growth in animals challenged with higher tumor burdens or using the highly aggressive B16-F10 variant [44,45]. In addition, vaccination with *N*AcGM3/VSSP after the surgical excision of primary tumors increased survival of melanoma-bearing mice [46].

Antitumor activity of *N*AcGM3/VSSP vaccine against melanoma was highly specific, adjuvant-dependent and non-transient. The vaccine induced a remarkable humoral response specific for GM3 ganglioside in mice, involving IgM as well as IgG class antibodies. Immunostaining and enzyme-linked immunosorbent assay (ELISA) experiments showed a high specificity of immune sera against GM3 and the presence of all four IgG subclasses, with preponderance of IgG2b and IgG3. In addition, a strong anti-B16 complement-mediated cytotoxicity was induced by vaccination with *N*AcGM3/VSSP [45]. The reactivity of serum IgG from vaccinated mice was confirmed by a sensitive immunoperoxidase assay on B16 specimens from tumor-bearing animals. Most melanoma cells displayed a distinct positive staining associated with both the cell membrane and cytoplasm [44]. In accordance with the immunohistochemical stainings, the antisera of immunized mice reacted strongly against B16 melanoma cells in flow cytometry studies. Recently, our group have shown that antitumor activity is associated with ganglioside expression on the tumor cell surface and demonstrated a major role of sialic acid in the humoral immunity of vaccinated mice [47].

Ganglioside immunotherapies with 1E10 antibody and molecular vaccines were well-tolerated in animals. In preclinical toxicology studies, the immunization protocol did not affect body weight gain, food and water consumption or induce other signs of overt toxicity in murine models. Subacute toxicity after continuous daily treatment was expressed by an excessive activation of extramedullary myelopoiesis in the spleen and liver in all mice and a strong inflammatory reaction in the lungs, showing dense neutrophil infiltrates in the interalveolar septa. Prior to Phase I clinical trials in humans, a 12-month dose-repeated study with the *N*AcGM3/VSSP vaccine was performed in *Macaca fascicularis* monkeys [48]. A total of 15 intramuscular vaccine injections were administered. Although the vaccine was strikingly immunogenic, maintaining unusually elevated anti-GM3 IgG and IgM titers throughout the study, no differences between treated and control monkeys were observed related to daily clinical observations, biochemical and hematological parameters and anti-DNA or antinuclear antibodies. Of the treated animals, 60% developed transient moderate local reaction at the injection site.

Clinical data

Tumor-specific expression of *N*euGc-containing gangliosides in some human tumors suggests that the induction of an effective immune response against these antigens may be useful for patients bearing antigen-positive tumors. Two Phase I clinical trials were conducted in patients with advanced malignant melanoma [41] and in patients with Stage III/IV breast cancer [49]. The patients were treated with repeated injections of aluminum hydroxide-precipitated 1E10 anti-idiotype antibody, given at 2-week intervals. The treatment was well-tolerated despite the development of antimouse Ig antibodies (HAMA response). It was noteworthy that most patients generated a very strong and specific anti-anti-idiotype antibody (Ab3)

response against *N*euGc-containing gangliosides, as detected by ELISA and HPTLC-immunostaining. This antigen-specific response was long lasting and the application of re-immunization to some patients provoked an increase in antibody titers. One interesting finding was the detection of a high level of binding of patient's hyperimmune sera to *N*GcGM3 ganglioside, after the complete abrogation of the reactivity against 1E10 monoclonal antibody by adsorbing the patient sera with this Ab2. The biological relevance of this nonclassical parallel set (Id^+Ag^+) remains to be elucidated.

Immunogenicity and toxicity profiles of the heterophilic ganglioside *N*GcGM3/VSSP/Montanide/ISA 51 vaccine were tested in advanced breast cancer patients [50]. 21 patients were included in a Phase I study and scheduled for biweekly intramuscular vaccination during the first 2 months of treatment (induction period) and monthly injections until the first year, to complete a total of 15 doses. The most frequent adverse effect was short-lasting pain in the injection site. Local skin reactions as induration, erythema, tenderness and swelling usually occurred at injection sites, which resolved within 1 to 3 days. Fever (grade I or II, according to WHO criteria) that disappeared spontaneously or by usual antipyretic treatment was also observed. After immunization, a high anti-*N*GcGM3 IgM antibody response was induced in all Stage III patients (maximum titer range from 1:10,240 to 1:164,000), while only half of Stage IV patients developed maximum titers greater than 1:10,000. High titer IgG-specific antibodies were also induced in 90% of Stage III patients (maximum titer range 1:10,240 to 1:164,000), while only in 33% of Stage IV patients serum titers were above 1:10,000. These data suggest that vaccine administration in the adjuvant setting may be more effective due to the observed higher immunogenicity induced in Stage III patients. Vaccination schedules including prolonged periods of re-immunization with the *N*GcGM3/VSSP preparation, even after disease progression, may be more adequate. In addition, a Phase I multicentric clinical trial with a ganglioside *N*AcGM3/VSSP/Montanide vaccine was conducted in advanced melanoma patients, using a similar regimen at three different dose levels. Although *N*AcGM3 is a ubiquitous ganglioside expressed in normal human tissues, the vaccine was reasonably well-tolerated [UNPUBLISHED OBSERVATIONS]. The most frequent adverse effects were pain, induration and erythema at the injection site, as well as fever and myalgias.

Currently, Phase II clinical trials with the *N*AcGM3 vaccine in disease-free advanced melanoma patients and with the *N*GcGM3 vaccine in Stage IV breast cancer patients, responders to first-line chemotherapy, are ongoing. In addition, a Phase II clinical trial with aluminum hydroxide-precipitated 1E10 anti-idiotype antibody in small cell lung cancer patients, responders to first-line chemotherapy, will be started, as well as a trial with the *N*AcGM3 vaccine in chronic lymphocytic leukemia. All these trials would add pivotal information about the value of the described approaches in the treatment of human cancer.

Conclusions

To date, ganglioside-based cancer vaccines have generally been restricted to melanoma and are still an area of active research. The unsuccessful GMK vaccine (Progenics Pharmaceuticals, NY, USA) targeting GM2 ganglioside [51] and the promising Canvaxin™ (CanVaxin, CA, USA) [52], a polyvalent melanoma cell vaccine composed of different gangliosides, are typical examples. In our current clinical program, the use of GM3 gangliosides-based vaccines and anti-idiotypic antibodies have been extended to other important pathologies such as breast, lung, colon and renal cancers and certain hematological malignancies also. Due to the different biological properties of these entities, the application of ganglioside vaccines to other tumors certainly presents a promising new opportunity in cancer therapeutics. Again, the main aspect for the selection of the appropriate target for ASI may be the biological role of a particular antigen in connection with tumor biology. In this sense, targeting a molecule such as *N*AcGM3 is very attractive, due to its strong immunosuppressive effects. The unique tumor-specific character of *N*GcGM3 allows us, for the first time, to consider tumor tissues as xenotransplants, presenting an interesting opportunity for antibodies. Vaccine design in the field of gangliosides, in which the starting point is to conserve their natural structures, may prove to be superior in the near future. This conception probably allows efficient stimulation of specific antiganglioside T-cell responses other than the classical humoral contribution. VSSP technology has proven to be an excellent way of fulfilling these expectations.

On the other hand, anti-idiotype antibodies in relation to *N*GcGM3 can induce the production of antiganglioside antibodies not only acting as classical antigen mimicry but also in exerting an immunomodulatory effect that activates a B-cell population different from that producing Id^+Ag^+ antibodies.

Expert opinion

Changes in the carbohydrate profile associated with malignant transformation and overexpression of gangliosides on the tumor cell surface are arguments to consider these molecules attractive targets for cancer immunotherapy. Recent clinical evidence showing a significant treatment benefit of high-dose α-interferon compared with the ganglioside vaccine GMK in high-risk melanoma patients has suggested serious limitations for ganglioside vaccines in cancer management [51]. However, it is important to note that cancer vaccines would be more effective in patients with minimal residual disease. Furthermore, comparison with proven anticancer therapies – such as interferon in melanoma patients – would not be the best approach to demonstrate the benefit of ganglioside immunotherapies. In the field of oncology, proper clinical trial planning is a critical issue in demonstrating the potential benefit of novel agents. A cancer vaccine should be designed as an additional treatment to standard therapy rather than an alternative to substitute treatment modalities with high toxicity. In this regard, despite improvements in surgical technique, radiotherapy and adjuvant chemotherapy, a significant proportion of patients apparently cured by surgery, subsequently relapse and die as a consequence of the disease.

The GMK vaccine was designed by conjugating GM2 ganglioside to KLH through a covalent bond and using QS-21 as adjuvant. Other formulations, such as the association with hydrophobic bacterial components or the use of anti-idiotypic antibodies, could favor better presentation of gangliosides to the immune system. GM3 is one of the most ubiquitous gangliosides and poorly immunogenic when administered as the unmodified nominal antigen. Therefore, even if tolerance to GM3 is difficult to break, when immunization is achieved, the antitumor response may be stronger. In this regard, induction of autoantibodies and tissue-specific autoimmunity against tumor cells represents an attractive approach to cancer immunotherapy [1].

Five-year view

Ganglioside-based immunotherapies, including molecular vaccines and anti-idiotype antibodies, are likely to be under intense investigation in clinical trials in the future. The completion of critical Phase II and III clinical trials will establish the real benefit of vaccination in melanoma, breast and lung cancer patients with residual disease who are at high risk of relapse. Basic research will provide a better understanding of the immune mechanisms of tumor rejection induced by ganglioside vaccines. In addition, preclinical animal models of cancer will be important in exploring synergic actions of vaccines in combination with other conventional cancer therapeutics. Rapid translation of relevant preclinical data into clinical trials will optimize the benefit in selected groups of cancer patients.

Potential conflicts of interest

DF Alonso and DE Gomez are consultants of Recom-Bio S.L., the company that is developing the 1E10 anti-idiotype antibody and *N*AcGM3 and *N*GcGM3 cancer vaccines and have been investigators during the preclinical testing of the products. No assistance with this review was provided by Recom-Bio.

Key issues

- Predictive animal models are required for preclinical testing of cancer vaccines and proper clinical trial planning.
- Although gangliosides are components of both normal and tumor cell membranes, improved vaccine formulations have shown to dramatically increase immunogenicity.
- Evidence of induction of direct immune responses against target cancer cells after immunization should be demonstrated in metastatic patients for each vaccine.
- The future development of ganglioside-based cancer vaccines and anti-idiotype antibodies will include expanded clinical trials to define the best patient population and therapeutic effectiveness.
- The application of vaccines in combination with other cancer therapies needs to be defined for each particular tumor variant.
- Although therapeutic cancer vaccines seem to be more effective in patients with minimal residual disease, evidences of objective responses in metastatic patients should be strongly validating.
- Highly sensitive molecular detection of circulating cancer cells based on RT-PCR will be evaluated as a potential end point of adjuvant vaccine therapy in clinical trials.

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