J Cancer Res Clin Oncol (2001) 127 (suppl 2): R20–R26

ORIGINAL PAPER

Cristina Musselli · Philip O. Livingston
Govindaswami Ragupathi

Keyhole limpet hemocyanin conjugate vaccines against cancer: the Memorial Sloan Kettering experience

Abstract Passively administered and actively induced antibodies have been associated with the eradication of circulating tumor cells and micrometastases in mice and humans. We have identified a series of cell surface carbohydrate and peptide antigens on melanomas, sarcomas, and cancer of the breast, prostate, ovary, and lung tissues. We found that breaking tolerance toward these tumor antigens was best achieved using vaccines containing antigens chemically conjugated to keyhole limpet hemocyanin (KLH) plus a potent immunological adjuvant (QS-21). To date, by using this approach to vaccination, antibodies have been induced in patients against glycolipid antigens GM2, GD2, GD3, FucosylGM1, Globo H, and Lewis Y, and glycoprotein (mucin) antigens Tn, sialyl Tn, TF, and MUC1. More recently, in a comparative study we investigated the T cell response induced by MUC1-KLH conjugates. Although a MUC1-specific T cell response was not consistently detected in any patient, the role of KLH in orienting the cytokine environment was crucial. We were able to confirm that KLH in these conjugate vaccines induces a Th1 T cell response as demonstrated by the high anti-KLH INF-γ secretion and the IgG1 and IgG3 subclasses of this high titer IgG antibodies induced. Clinical trials using KLH conjugated glycolipid and glycoprotein vaccines, are currently ongoing. These range from phase I/II single antigens trials with glycosilated MUC1, polysialic acid, synthetic Fucosyl GM1 and GD2, to phase II trials with a polyvalent vaccine containing six or seven antigens. Randomized phase II trials with polyvalent vaccines are planned for initiation in 2001–2002 in patients with ovarian, breast, and prostate cancer.

Work presented at the 24th Congress of the German Cancer Society: Keyhole limpet hemocyanin (KLH) conjugate vaccines as novel therapeutic tools in malignant disorders. 20 March 2000, Berlin, Germany.

C. Musselli · P. O. Livingston · G. Ragupathi
Memorial Sloan Kettering Cancer Center,
Department of Tumor Vaccinology,
1275 York Avenue, New York, NY 10021, USA

Introduction

Since Edward Jenner carried out the first successful human vaccination some 200 years ago, the effort of vaccinologists has been directed primarily toward the preparation of vaccines able to elicit protective antibodies. In the 1950s and 1960s a series of elegant studies [8, 46, 11, 34] showed that carcinogen- or virally induced experimental tumors were highly immunogenic in syngeneic mice and rats. This notion assimilated cancer to infectious diseases in that a specific immune response was evocable. Microorganisms may possess hundreds of molecules that are foreign to a host, and are thus antigenic, but an immune response against the majority of these is entirely irrelevant to the prevention of infection. Cancer cells share this property, with the addition that cancer cells arise from self and so their antigens are tolerated to a greater or lesser degree. To circumvent these problems, increasing attention is being given to the identification and purification or synthesis of protective antigens and their association with a carrier and/or an immunological adjuvant for optimal antibody induction [12–14]. Most practical vaccine development programs against infectious diseases have relied on the vaccine's capacity to evoke antibody formation but they can elicit other kinds of immune response, including T helper 1 (Th1)- and T helper 2 (Th2)-type CD4 T cells, or CD8 T cell responses. The same range of immune responses applies to vaccine development programs against cancer, and active and passive antibody administration has been shown to eradicate tumor in experimental animals and in humans [6, 16, 18, 17, 42, 38, 31, 27, 47, 30, 19]. Here we review our experience in breaking tolerance to various tumor antigens by keyhole limpet hemocyanin (KLH)-conjugates in association with the use of the potent immune adjuvant QS-21 in the adjuvant setting of cancer therapy.

Early trials with gangliosides

During the late 1970s and 1980s, Livingston et al. [24, 26] and Tai et al. [45] identified gangliosides as tumor molecules potentially able to induce an immune response after vaccination. By immunizing mice and patients with whole melanoma cells or cell lysates [29, 28, 25, 23], GM2 and GD2 were identified as responsible for the induction of IgM antibodies at low but detectable titers with a short half-life. Nevertheless, gangliosides were identified as targets for immunotherapy. This led to their purification and synthesis after which several vaccine strategies were explored in the 1980s. GM2 alone did not induce any antibody response, therefore the immunogenicity of GM2 plus *Salmonella minnesota* R595, BCG or liposome with MPLA were compared in mice and humans. These studies indicated that while *Salmonella* was better in mice [24], only BCG was able to induce consistent IgM antibodies in humans at a moderate and short-lived titer. However, there was no secondary response after boost. Patients with antibodies against GM2 after vaccination were found to have a longer disease-free and overall survival [26]. Therefore, a randomized trial was initiated to prove the efficacy of GM2 plus BCG in comparison to BCG alone. One hundred and twenty-two stage III melanoma patients were enrolled and it was shown that the antibody response was induced in patients receiving GM2 and not in the controls. By FACS, these elicited IgM were able to bind to tumor cells expressing the antigen. Furthermore, these antibodies were able to mediate CDC [30, 22]. Once again, patients with natural or vaccine-induced antibodies against GM2 had a significantly prolonged disease-free and overall survival compared to antibody-negative patients. Comparing the GM2/BCG and BCG groups as randomized there was no significant difference in disease-free or overall survival, though there was a strong trend in favor of the GM2/BCG group. Trials were initiated with GD2/BCG and GD3/BCG. Antibodies were induced in occasional patients immunized with GD2 but not with GD3. Clearly, the door to immunization against melanoma antigens was open, but the necessity to make it more efficient was a priority. Two approaches were taken: i) modification of ganglioside structure (GD3 lactone, GD3 amide, GD3 gangliosidol-O- acetyl GD3) that failed to induce antibody against unmodified GD3 [39]; or ii) conjugating GD3 to a carrier.

KLH-conjugation and gangliosides: the prototype

In 1994 Helling et al. [13] showed the ability of conjugation to a carrier protein to improve the immunogenicity of a tumor antigen and bolster an otherwise weak antibody response. To induce a strong antibody response against the antigen of interest, the optimal strategy is to conjugate the antigen to a carrier and vaccinate in combination with a strong immune adjuvant (Fig. 1). Helling compared different carriers conjugated to GD3 in an attempt to obtain the highest titer anti-GD3 antibodies. The comparison included a series of different carrier molecules, such as: poly-L-lysine hydrobromide for the most intense Ag epitope expression; bovine serum albumin (BSA) imject super-carrier immune modulator which was modified to express a quite intense number of epitopes; *Neisseria meningitidis* outer membrane proteins (OMP) which have been widely used in vaccines against infectious diseases; multiple antigen peptide (MAP YAL-IV 294-I) contained four repeats of the malarial T-cell epitope and possesses branches so that it guarantees a multiplication of epitopes; ABH (p-azidobenzoyl-hydrozyde) because it is able to link carbohydrate to proteins; and finally, KLH (keyhole limpet hemocyanin), a big protein (5×10^6 D) extracted from keyhole limpets. Conjugation with KLH proved superior to all the others in mice immunized with GD3 – KLH + QS-21, resulting in long-lasting production of both IgM and IgG in high titer [13, 12]. The path for the first clinical trial with conjugate GM2-KLH was open and studies commenced in humans to compare a large variety of different adjuvants. QS-21, a purified saponin fraction separated from the bark of *Quillaja saponaria* was optimal. Trials in humans demonstrated once again that the use of QS-21 led to the secretion of the highest titers of IgM and IgG [14]. Antibodies were able to recognize GM2 on the surface of tumor cells by FACS and were functionally active in inducing tumor killing through complement-(CDC) and antibody-dependent cellular cytotoxicity (ADCC) [14, 32].

Conjugate Vaccine Components for Optimal Antibody Induction

Antigen	—	Carrier (KLH)	+	Adjuvant (QS-21)
Antigen configuration must mimic expression on tumor cell		Cytokine release proportional to carrier immunogenicity		Activation of APCs, B-cells and T-cells
High antigen/carrier ratio needed		Cytokine release sequence may be key		Depot effect

Fig. 1 Scheme of vaccine constructs used with all the different tumor antigens

Focusing on antigen identification

Between 1993 and 1997, by using available monoclonal antibodies, a series of immunohistochemistry studies were performed [51, 48, 50]. These studies allowed the identification of other antigens expressed on the surface of different tumors, generating candidates for KLH

Table 1 Antigen tested in immunohistologic screen

Gangliosides	Blood related	Proteins
GD3 GD2 GM2	Tn sTn TF	MUC1 2 3 4 7
9–0-Acetyl GD3	Globo H	MUC 5ac MUC 5b
Fucosyl GM1	Lea Sialyl Lea	CEA
Polysialic acid	Ley Leb	PSMA
	Lex Sialyl Lex	KSA
	Polyfucosyl Lex	HCGb

conjugate vaccines (Table 1). These molecules can be defined in three categories: i) gangliosides, selectively expressed on melanoma, sarcoma, or neuroblastoma with the exception of GM2 which is widely expressed in all tumor cells tested; ii) blood-group related antigens or glycolipid antigens expressed on a wide variety of epithelial cancers (breast, prostate, lung, colon, pancreas, ovary); and iii) proteins, expressed again on tumors of epithelial origin. These molecules are expressed on the surface of tumor cells protruding up to 100 Å from the cell membrane as glycolipid or as glycoproteins and mucins with their associated carbohydrates (Tn, sTn, TF) forming the "mucin layer" a glycocalix which extends up to 4,000 Å from the cell surface (Fig. 2). Each of these classes of surface antigens represent good targets for humoral or cellular response.

KLH-Conjugates in clinical trials

After tests on a range of normal and malignant tissues, thirteen of the 28 antigens shown in Table 1 were selected as suitable targets and conjugated to KLH, mixed with QS-21, and tested in mice as well as in a series of phase-I clinical trials. Table 2 shows a summary of the results obtained in some of these trials. Three groups of patient responses can be identified based on ELISA responses against the synthetic antigens and reaction with tumor cells: high titer, universal responders to sTn, GM2 [30], and FucosylGM1 [7]; intermediate titer responders to Globo H [43, 10], Tn, and MUC1 [9] (breast and prostate); and low titer responders to GD2 [5, 4],

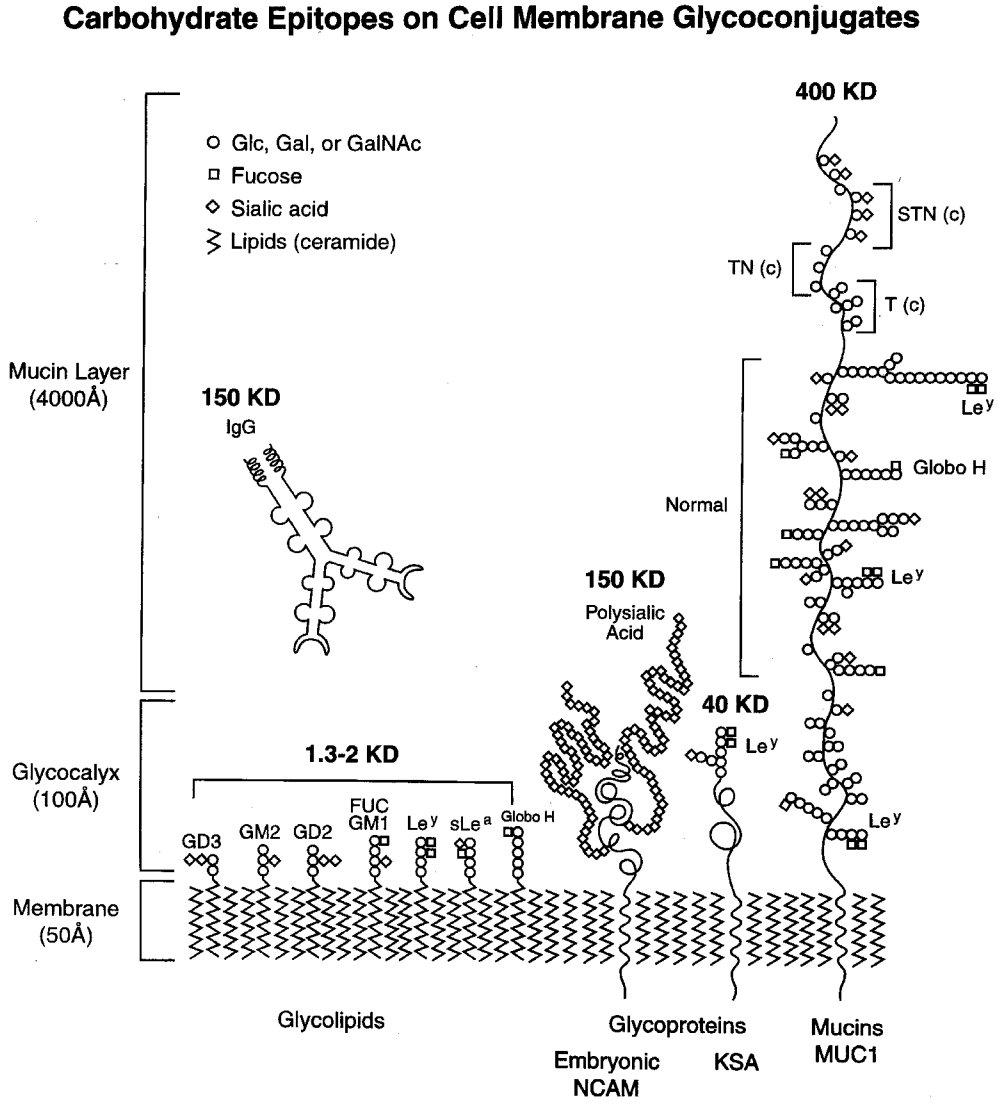

Fig. 2 Representation of steric conformation of the different tumor antigens on the cell surface of tumor cells

Table 2 Summary of serological results in vaccinated patients

Antigen	Median ELISA		IgG subclass	Median FACS		Median IA	Median CDC
	IgM	IgG		IgM	IgG		
GM2	640	320	IgG1+3	3+	2+	2+	2+
GD3	80	160		1+	1+	1+	1+
Fucosyl GM1	320	640	IgG1	3+	2+		2+
Globo H	640	40	IgG1+3	2+	1+	2+	1+
Lewis Y	80	0		2+	1+	2+	1+
Tn	1280	1280		2+	neg	1+	neg
sTn	1280	160	IgG3	3+	neg	1+	neg
TF	320	10			neg	1+	neg
MUC1	1280	5120	IgG1+3	1+	neg	neg	neg
KSA	40	160		neg	neg	neg	neg
Polysialic acid	480	0		3+	neg		neg

GD3 [35], LeY [41], and TF (melanoma, ovarian, and prostate). The overall response was essentially 100% for patients immunized with GM2, FucosylGM1, and sTN, and between 60% and 80% for patients immunized with GD3-lactone, Globo H, n-propionyl-polysialic acid, polysialic acid, TN, and MUC1, and between 40% to 60% for patients immunized with GD2-lactone, LeY, and TF. Serologic responses against MUC2 and KSA have not yet been shown to react with tumor cells.

Globo H may serve as an example of our experience with glycolipid antigens. Globo H was originally identified by Hakamori et al. [20, 3] with the monoclonal antibody MBr1 [33] and subsequently synthesized by Danishefsky et al. [2] in our group. It is expressed on a variety of epithelial cancers and is utilized in our breast and prostate cancer vaccines. Proper synthesis is critical as shown by binding inhibition studies with MBr1. It is in fact sufficient to introduce a simple change in its structure, from a β to a α bond between the third and fourth sugar, to completely abrogate the binding. The same kind of study was performed on the sera of patients immunized with Globo H. In this case, sera are inhibited by Globo H and not by GD3, but we do see some inhibition with structures that share at least four of the same sugars with Globo H, suggesting the induction of a polyvalent antibody response [37, 36]. In clinical trials the use of Globo H – KLH conjugate in combination with QS-21, resulted in a tumor-specific antibody response by ELISA and FACS (Fig. 3 and Fig. 4) that was capable of mediating strong CDC and ADCC [43, 10]. In some cases, this response lasted up to 2 years after the therapy without any further boost.

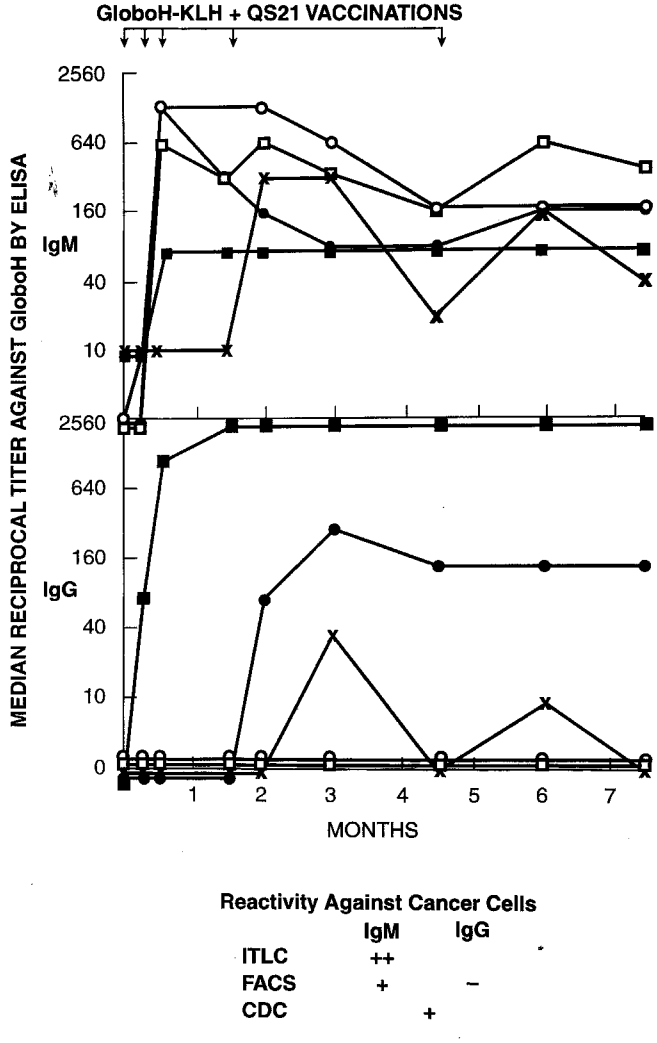

Fig. 3 Antibody titer by Elisa in patients vaccinated with Globo H-KLH plus adjuvant QS-21

Mucins and other glycoproteins

Our experience with MUC1 is typical of our experience with mucin antigens. Mucins are highly glycosilated proteins expressed at the lumenal side of epithelial cells where they may protect the mucosae. They are also expressed on tumor cells of the same origin, with MUC1 being the best known. In tumor cells, mucins may be over expressed and under glycosilated [1]. These properties make MUC1 a potential target for cancer immunotherapy, so different approaches to its conjugation with KLH were explored. In this case, a different method was applied, using the linker molecule MBS between the antigen MUC1 and the carrier KLH [49]. This resulted in several trials in breast and prostate cancer, where we

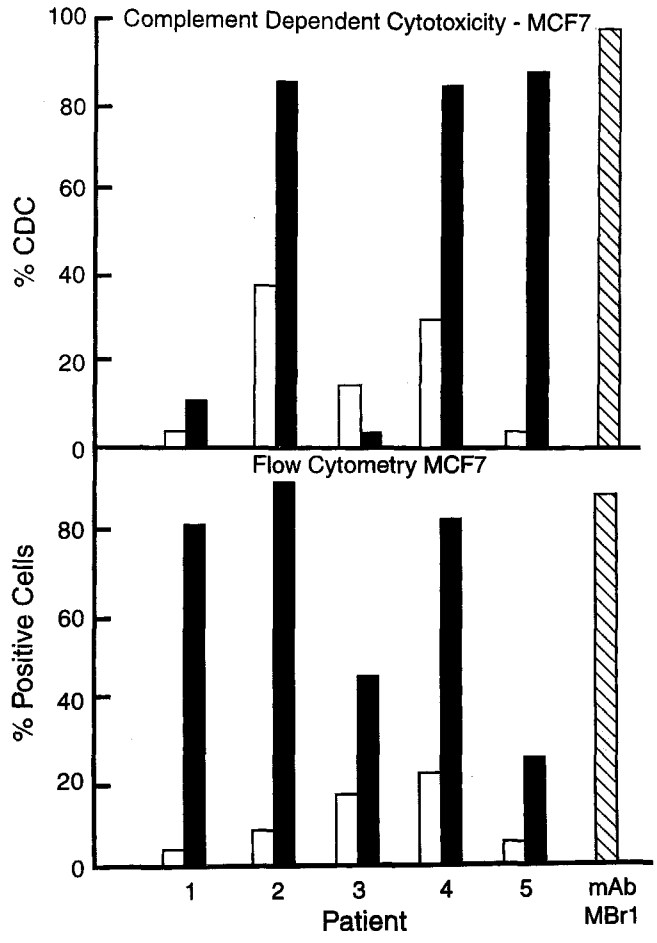

Fig. 4 Cell surface binding (FACS) and biological activity (CDC) of sera pre- and post-immunization in patients vaccinated with Globo H-KLK plus adjuvant QS-21

were able to show a high titer of specific antibodies that recognize both the synthetic antigen in ELISA as well as the native form on the cell surface by FACS [9]. Interestingly, these antibodies did not mediate CDC (Liu N et al., manuscript in preparation), but may be effective at inducing ADCC [44].

We are currently evaluating other glycoprotein antigens including KSA, PSMA, and melanosomal antigens chemically conjugated with KLH in murine systems.

How about T cells?

KLH-conjugates have been shown to induce a strong, consistent, long-lasting, tumor-specific antibody response. The isotype of these antibodies is both IgM and IgG, mainly IgG1 and IgG3. This profile indicates the involvement of T cells, specifically helper CD4 of the Th1 subtype. Therefore, we recently initiated some experiments to evaluate the capability of the conjugates to induce a T cell response. We utilized spleen cells from mice immunized with MUC1-KLH plus QS-21, and peripheral blood mononuclear cells (PBMC) from breast

Table 3 Stimulation index, IFN-γ, and IL-4 secretion induced by 96 h in vitro stimulation after mice vaccination with MUC1-KLH plus adjuvant IFN-γ and IL-4 are expressed as pg/ml

Adjuvant	In vitro stimulation antigen					
	KLH			MUC1		
	S.I.	IFN-γ	IL4	S.I.	IFNγ	IL4
QS-21	11	6573	683	1.2	222	166
MPL-SE	8.3	5233	406	1.5	1121	114
MoGM-CSF	6.9	1164	637	1.4	367	96
Detox-PC	5.7	nd	186	0.9	nd	86
TiterMax-G	2.7	694	30	1.6	66	71
Saline	1.2	181	75	1.1	171	101
GpG ODN	1.1	9602	142	1	1158	29

Table 4 Cytokine profile on supernatants of KLH unstimulated or stimulated post-immune human T cells after 6 days of culture

Patients	Cell type	IL4 pg/ml		INFγ U/ml	
		Unstimulated	Stimulated	Unstimulated	Stimulated
10	PBMC	25	27	0	28
11	PBMC	24	26	0	22
13	PBMC	34	30	0	26
1	CD4	78	75	2	49
12	CD4	51	53	1	25
	CD8	91	99	3	4
16	CD4	253	215	7	36
	CD8	110	137	5	18
18	CD4	71	96	14	35
	CD8	272	185	41	25

Table 5 Proliferation assay and IFN-γ Elispot - human T cell response to KLH and MUC1 after 6 days of in vitro stimulation. Data are expressed as mean value of six patients tested in *24 and **11 tests respectively. Spots are over 100,000 PBMC

Antigen	Pre-immune		Post-immune	
	S.I.*	Spots**	S.I.*	Spots**
KLH	3,2	96	106	307
MUC1	1,1	28	3,2	88
PHA	89	1127	118	1078

cancer patients vaccinated with the same construct. In both mouse and human, whole T cells stimulated in vitro with KLH preferentially secrete INFγ at high levels IL4 (Table 3). Moreover in humans, purified CD4 T cells isolated from PBMC of vaccinated patients secrete INFγ in response to KLH, with undetectable IL4. These data confirm the crucial role of KLH in determining a Th1 profile in the immune response (Table 4). This result was further observed in a comparative study in the mouse, where we used 20 different adjuvants and INFγ was secreted at a higher level with respect to IL-4 in response to KLH, regardless of adjuvant [21]. Proliferation and Elispot assays were used to analyze the response to MUC1 tumor antigen. As shown in Table 5, a strong T

cell response to the foreign protein KLH was observed in both assays. Less consistent was the T cell response to MUC1 peptide. A sporadic stimulation after immunization was observed in proliferation assays, and by the single-cell analysis of INFγ Elispot [52]. Both assays were performed after 6 days of stimulation (Table 5). Moreover, no DTH was observed in patients immunized with MUC1 or any of the other antigens. These data confirm the ability of KLH to strongly drive the antibody response against the conjugated antigens toward a cytotoxic Th1 profile.

Future plans

Tumor cells express many antigens on their surface. This tumor heterogeneity and heterogeneity of the human immune response strongly support the use of polyvalent vaccines. Some possible targets for this strategy are gangliosides GM2, GD2, and GD3 for melanoma, neuroblastoma, and sarcoma; gangliosides, Globo H, polysialic acid, and Fucosyl GM1 for small cell lung cancer; GM2, mucin-related carbohydrates, Lewis Y and Globo H for breast, prostate, and ovary. In this regard, randomized adjuvant phase II trials are to be initiated at MSKCC with polyvalent-KLH-QS-21 vaccines in patients with each of these cancers over the next 2 years.

KLH conjugation has been shown to be the best-known strategy for antibody induction in cancer patients. Randomized trials with polyvalent KLH-conjugate plus QS-21 vaccines will determine the impact of this approach on disease-free and overall survival in the adjuvant setting. On the other hand, the optimal vaccination approach for inducing T cell responses against cancer antigens remains to be determined. Our group is exploring a broad range of approaches toward this end, ranging from KLH conjugation with whole proteins and peptides, to the same peptide and proteins unconjugated, to DNA or viral vector vaccines expressing the genes for these peptides and proteins [40, 15].

References

1. Barrat-Boyes S (1996) Making the most of mucin: a novel target for immunotherapy. Cancer Immunol Immunother 43: 142–151
2. Bilodeau MT, Park TK, Hu S, Randolph JT, Danishefsky SJ, Livingston PO, Zhang S (1995) Total synthesis of a human breast tumor-associated antigen. J Am Chem Soc 117: 7840–7841
3. Bremer EG, Levery SB, Sonnino S, Ghidoni R, Canevari S, Kannagi R, Hakomori S (1984) Characterization of a glycosphingolipid antigen defined by the monoclonal antibody MBr1 expressed in normal and neoplastic epithelial cells of human mammary gland. J Biol Chem 259: 14773–14777
4. Chapman PB, Morrisey D, Panageas KS, Williams L, Lewis JJ, Israel RJ, Hamilton WB, Livingston PO (2000) Vaccination with a bivalent G(M2) and G(D2) ganglioside conjugate vaccine: a trial comparing doses of G(D2)-keyhole limpet hemocyanin. Clin Cancer Res 6: 4658–4662
5. Chapman PB, Morrissey DM, Panageas KS, Hamilton WB, Zhan C, Destro AN, Williams L, Israel RJ, Livingston PO (2000) Induction of antibodies against GM2 ganglioside by immunizing melanoma patients using GM2-keyhole limpet hemocyanin + QS21 vaccine: a dose-response study. Clin Cancer Res 6: 874–879
6. Cheung NK, Lazarus H, Miraldi FD, Abramowsky CR, Kallick S, Saarinen UM, Spitzer T, Strandjord SE, Coccia PF, Berger NA (1987) Ganglioside GD2-specific monoclonal antibody 3F8: a phase I study in patients with neuroblastoma and malignant melanoma. J Clin Oncol 5: 1430–1440
7. Dickler MN, Ragupathi G, Liu NX, Musselli C, Martino DJ, Miller VA, Kris MG, Brezicka FT, Livingston PO, Grant SC (1999) Immunogenicity of a fucosyl-GM1-keyhole limpet hemocyanin conjugate vaccine in patients with small cell lung cancer. Clin Cancer Res 5: 2773–2779
8. Ehrlich P (1909) Uber den jetzigen Stand der Karzinomforschung. Ned Tijdschr Geneeskd 5: 273–290
9. Gilewski T, Adluri S, Ragupathi G, Zhang S, Yao TJ, Panageas K, Moynahan M, Houghton A, Norton L, Livingston PO (2000) Vaccination of high-risk breast cancer patients with mucin-1 (MUC1) keyhole limpet hemocyanin conjugate plus QS-21. Clin Cancer Res 6: 1693–1701
10. Gilewski T, Ragupathi G, Williams LJ, Musselli C, Zhang XF, Bencsath KP, Panageas KS, Chin J, Hudis CA, Norton L, Houghton AN, Livingston PO, Danishefsky SJ (2001) Immunization of metastatic breast cancer patients with a fully synthetic Globo H conjugate: a phase I trial. Proc Natl Acad Sci USA 98: 3270–3275
11. Gorer P (1956) Some recent work on tumor immunity. Adv Cancer Res 4: 149–186
12. Helling F, Livingston PO (1994) Ganglioside conjugate vaccines. Immunotherapy against tumors of neuroectodermal origin. Mol Chem Neuropathol 21: 299–309
13. Helling F, Shang A, Calves M, Zhang S, Ren S, Yu RK, Oettgen HF, Livingston PO (1994) GD3 vaccines for melanoma: superior immunogenicity of keyhole limpet hemocyanin conjugate vaccines. Cancer Res 54: 197–203
14. Helling F, Zhang S, Shang A, Adluri S, Calves M, Koganty R, Longenecker BM, Yao TJ, Oettgen HF, Livingston PO (1995) GM2-KLH conjugate vaccine: increased immunogenicity in melanoma patients after administration with immunological adjuvant QS-21. Cancer Res 55: 2783–2788
15. Hirschowitz EA, Leonard S, Song W, Ferris B, Leopold PL, Lewis JJ, Bowne WB, Wang S, Houghton AN, Crystal RG (1998) Adenovirus-mediated expression of melanoma antigen gp75 as immunotherapy for metastatic melanoma. Gene Ther 5: 975–983
16. Houghton AN, Mintzer D, Cordon-Cardo C, Welt S, Fliegel B, Vadhan S, Carswell E, Melamed MR, Oettgen HF, Old LJ (1985) Mouse monoclonal IgG3 antibody detecting GD3 ganglioside: a phase I trial in patients with malignant melanoma. Proc Natl Acad Sci USA 82: 1242–1246
17. Irie RF, Matsuki T, Morton DL (1989) Human monoclonal antibody to ganglioside GM2 for melanoma treatment. Lancet 1: 786–787
18. Irie RF, Morton DL (1986) Regression of cutaneous metastatic melanoma by intralesional injection with human monoclonal antibody to ganglioside GD2. Proc Natl Acad Sci USA 83: 8694–8698
19. Jones PC, Sze LL, Liu PY, Morton DL, Irie RF (1981) Prolonged survival for melanoma patients with elevated IgM antibody to oncofetal antigen. J Natl Cancer Inst 66: 249–254
20. Kannagi R, Levery SB, Ishigami F, Hakomori S, Shevinsky LH, Knowles BB, Solter D (1983) New globoseries glycosphingolipids in human teratocarcinoma reactive with the monoclonal antibody directed to a developmentally regulated antigen, stage-specific embryonic antigen 3. J Biol Chem 258: 8934–8942
21. Kim SK, Ragupathi G, Musselli C, Choi SJ, Park YS, Livingston PO (1999) Comparison of the effect of different immunological adjuvants on the antibody and T-cell response to

immunization with MUC1-KLH and GD3-KLH conjugate cancer vaccines. Vaccine 18: 597–603
22. Livingston PO, Adluri S, Helling F, Yao TJ, Kensil CR, Newman MJ, Marciani D (1994) Phase 1 trial of immunological adjuvant QS-21 with a GM2 ganglioside-keyhole limpet haemocyanin conjugate vaccine in patients with malignant melanoma. Vaccine 12: 1275–1280
23. Livingston PO, Albino AP, Chung TJ, Real FX, Houghton AN, Oettgen HF, Old LJ (1985) Serological response of melanoma patients to vaccines prepared from VSV lysates of autologous and allogeneic-cultured melanoma cells. Cancer 55: 713–720
24. Livingston PO, Calves MJ, Natoli EJ (1987) Approaches to augmenting the immunogenicity of the ganglioside GM2 in mice: purified GM2 is superior to whole cells. J Immunol 138: 1524–1529
25. Livingston PO, DeLeo AB, Jones M, Oettgen HF (1983) Comparison of approaches for augmenting the serologic response to the individually specific methylcholanthrene-induced sarcoma-Meth A: pretreatment with cyclophosphamide is most effective. J Immunol 131: 2601–2605
26. Livingston PO, Natoli EJ, Calves MJ, Stockert E, Oettgen HF, Old LJ (1987) Vaccines containing purified GM2 ganglioside elicit GM2 antibodies in melanoma patients. Proc Natl Acad Sci USA 84: 2911–2915
27. Livingston PO, Ragupathi G (1997) Carbohydrate vaccines that induce antibodies against cancer. 2. Previous experience and future plans. Cancer Immunol Immunother 45: 10–19
28. Livingston PO, Takeyama H, Pollack MS, Houghton AN, Albino A, Pinsky CM, Oettgen HF, Old LJ (1983) Serological responses of melanoma patients to vaccines derived from allogeneic cultured melanoma cells. Int J Cancer 31: 567–575
29. Livingston PO, Watanabe T, Shiku H, Houghton AN, Albino A, Takahashi T, Resnick LA, Michitsch R, Pinsky CM, Oettgen HF, Old LJ (1982) Serological response of melanoma patients receiving melanoma cell vaccines. I. Autologous cultured melanoma cells. Int J Cancer 30: 413–422
30. Livingston PO, Wong GY, Adluri S, Tao Y, Padavan M, Parente R, Hanlon C, Calves MJ, Helling F, Ritter G, et al (1994) Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. J Clin Oncol 12: 1036–1044
31. Livingston PO, Zhang S, Lloyd KO (1997) Carbohydrate vaccines that induce antibodies against cancer. 1. Rationale. Cancer Immunol Immunother 45: 1–9
32. Livingston PO, Zhang S, Adluri S, Yao TJ, Graeber L, Ragupathi G, Helling H, Fleisher M (1997) Tumor cell reactivity mediated by IgM antibodies in sera from melanoma patients vaccinated with GM2 ganglioside covalently linked to KLH is increased by IgG antibodies. Cancer Immunol Immunother 43: 324–330
33. Menard S, Tagliabue E, Canevari S, Fossati G, Colnaghi MI (1983) Generation of monoclonal antibodies reacting with normal and cancer cells of human breast. Cancer Res 43: 1295–1300
34. Old LJ, Boyse EA, Clarke DA, Carswell EA (1962) Antigenic properties of chemically induced tumors. Ann NY Acad Sci 101: 80–106
35. Ragupathi G, Meyers M, Adluri S, Howard L, Musselli C, Livingston PO (2000) Induction of antibodies against GD3 ganglioside in melanoma patients by vaccination with GD3-lactone-KLH conjugate plus immunological adjuvant QS-21. Int J Cancer 85: 659–666
36. Ragupathi G, Slovin SF, Adluri S, Sames D, Kim IJ, Kim HM, Spassova M, Bornmann WG, Lloyd KO, Scher HI, Livingston PO, Danishefsky SJ (1999) A fully synthetic Globo H carbohydrate vaccine induces a focused humoral response in prostate cancer patients: a proof of principle. Angew Chem Int Ed 38: 563–566
37. Ragupathi GPT, Zhang S, Kim IJ, Graber L, Adluri S, Lloyd KO, Danishefsky SJ, Livingston PO (1997) Immunization of mice with a fully synthetic Globo H antigen results in antibodies against human cancer cells: a combined chemical-immunological approach to the fashioning of an anticancer vaccine. Angew Chem Int Ed Engl 36: 125–128
38. Riethmuller G, Schneider-Gadicke E, Schlimok G, Schmiegel W, Raab R, Hoffken K, Gruber R, Pichlmaier H, Hirche H, Pichlmayr R, et al (1994) Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. German Cancer Aid 17–1A Study Group. Lancet 343: 1177–1183
39. Ritter G, Boosfeld E, Adluri R, Calves M, Oettgen HF, Old LJ, Livingston P (1991) Antibody response to immunization with ganglioside GD3 and GD3 congeners (lactones, amide and gangliosidol) in patients with malignant melanoma. Int J Cancer 48: 379–385
40. Ross HM, Weber LW, Wang S, Piskun G, Dyall R, Song P, Takechi Y, Nikolic-Zugic J, Houghton AN, Lewis JJ (1997) Priming for T-cell-mediated rejection of established tumors by cutaneous DNA immunization. Clin Cancer Res 3: 2191–2196
41. Sabbatini PJ, Kudryashov V, Ragupathi G, Danishefsky SJ, Livingston PO, Bornmann W, Spassova M, Zatorski A, Spriggs D, Aghajanian C, Soignet S, Peyton M, O'Flaherty C, Curtin J, Lloyd KO (2000) Immunization of ovarian cancer patients with a synthetic Lewis(y)-protein conjugate vaccine: a phase 1 trial. Int J Cancer 87: 79–85
42. Saleh MN, Khazaeli MB, Wheeler RH, Dropcho E, Liu T, Urist M, Miller DM, Lawson S, Dixon P, Russell CH, et al (1992) Phase I trial of the murine monoclonal anti-GD2 antibody 14G2a in metastatic melanoma. Cancer Res 52: 4342–4347
43. Slovin SF, Ragupathi G, Adluri S, Ungers G, Terry K, Kim S, Spassova M, Bornmann WG, Fazzari M, Dantis L, Olkiewicz K, Lloyd KO, Livingston PO, Danishefsky SJ, Scher HI (1999) Carbohydrate vaccines in cancer: immunogenicity of a fully synthetic Globo H hexasaccharide conjugate in man. Proc Natl Acad Sci USA 96: 5710–5715
44. Snijdewint FGM, von Mensdorff-Pouilly S, Karuntu-Wanamarta AH, Verstraeten AA, Livingston PO, Kenemas P (2001) Antibody-dependent cell-mediated cytotoxicity can be induced by MUC1 peptide vaccination of breast cancer patients. Cancer Immunol Immunother 48: 47–55
45. Tai T, Cahan LD, Tsuchida T, Saxton RE, Irie RF, Morton DL (1985) Immunogenicity of melanoma-associated gangliosides in cancer patients. Int J Cancer 35: 607–612
46. Woglom W (1929) Immunity to transplantable tumors. Cancer Rev 4: 129–214
47. Zhang H, Zhang S, Cheung NK, Ragupathi G, Livingston PO (1998) Antibodies against GD2 ganglioside can eradicate syngeneic cancer micrometastases. Cancer Res 58: 2844–2849
48. Zhang S, Cordon-Cardo C, Zhang HS, Reuter VE, Adluri S, Hamilton WB, Lloyd KO, Livingston PO (1997) Selection of tumor antigens as targets for immune attack using immunohistochemistry: I. Focus on gangliosides. Int J Cancer 73: 42–49
49. Zhang S, Graeber LA, Helling F, Ragupathi G, Adluri S, Lloyd KO, Livingston PO (1996) Augmenting the immunogenicity of synthetic MUC1 peptide vaccines in mice. Cancer Res 56: 3315–3319
50. Zhang S, Zhang HS, Cordon-Cardo C, Ragupathi G, Livingston PO (1998) Selection of tumor antigens as targets for immune attack using immunohistochemistry: protein antigens. Clin Cancer Res 4: 2669–2676
51. Zhang S, Zhang HS, Cordon-Cardo C, Reuter VE, Singhal AK, Lloyd KO, Livingston PO (1997) Selection of tumor antigens as targets for immune attack using immunohistochemistry: II. Blood group-related antigens. Int J Cancer 73: 50–56
52. Musselli G, Ragupathi G, Gilewski T, Panageas KS, Spinat Y, Livingston PO (2001) Re-evaluation of the cellular immune response in breast cancer patients vaccinated with MUC1. Int J Cancer (in press)