that paralell our human clinical studies. Some of the mice that had recieved recombinant viruses expressing the murine versions of the human tumor rejection antigens had experienced coat color changes (from black to white) that were very similar to the vitiligo that we see in some of our responding patients.

WHAT DO GANGLIOSIDES SHOW US ABOUT IDIOTYPIC NETWORKS?

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Introduction

Tumor-associated gangliosides are carbohydrate self-antigens very poorly immunogenic (1). Many attempts have been made trying to improve their immunogenicity by using T cell dependent vectors. However, specific active immunotherapy with tumor-associated gangliosides has not been successful enough in the clinical setting (2). We had the hypothesis that anti-idiotypic MAbs could be used as T cell dependent antigen surrogates, as it has been suggested by others (3).

Results and Discussion

Anti-Ganglioside Antibodies are most likely natural autoantibodies

Liposomes containing GM3 (NeuGc) elicited non boosteable, low titer, cross-reactive anti-ganglioside IgM antibody responses in Balb/c mice. A unique IgM MAb, P3, highly specific for N-glycolylated gangliosides was obtained (4). P3 MAb heavy chain variable region had 94 % homology with an anti-PC Ab, using the Q52 VH gene family (5), which is over-represented in neonatal "natural" antibodies characterized by high degree of degenerate "specificity" and high idiotypic connectivity (6). Two N-terminal addition segments contribute to the H3 diversity of MAb P3. An Arginine pocket could be defined on the P3 heavy chain variable region surface, into which the negative carboxyl group of sialic acid might dock.

Anti-Ganglioside Antibodies Induce an Antigen-Independent Natural Autoantibody response

This Ab1 antibody (P3), as other anti-ganglioside IgM antibodies coupled with KLH, elicited a multispecific anti-ganglioside IgM antibody serological responses (Ab1', titers 1/160 to 1/640) in syngeneic models. Clonal analysis of this anti-anti-idiotypic responses by cell fusion experiments showed the multispecificity of such Abs (reactivity also with N-acetylated gangliosides). Ab1' responses had a maximun after two immunization doses, while dropped with a third dose.

Anti-Ganglioside Antibodies Induce An Strong Anti-Idiotypic Response

Anti-ganglioside IgM antibodies like P3 MAb coupled with KLH raised strong IgG anti-idiotypic responses (Ab2, titers 1/10 000 to 1/50 000) in Balb/c mice. No correlation between the IgM Ab1' and the IgG Ab2 titers was found, but 98 % of Ab2 clones were specific for P3 MAb after the second dose, and only 68 % after the third one. Moreover, most of these specific Ab2 clones were able to block binding of P3 MAb to GM3 (NeuGc). The heavy chain variable region sequence of one paratopic Ab2 contained 2 and 4 Asp residues into H2 and H3 respectively, suggesting an "interaction mimicry" of the sialic acid negative charge.

Anti-Idiotypic Antibodies Induce a "Natural" Autoantibody response to Gangliosides

Half of the paratopic Ab2s tested so far *in vivo*, coupled with KLH and combined with Freund incomplete adjuvant induced a low titer, not boosteable, multispecific IgM antibody serological responses in syngeneic and allogeneic models.

Concluding Remarks

In general, anti-Id MAbs to primary response anti-ganglioside MAbs do not behave as T cell dependent antigen surrogates. To change the chemical nature of the antigen surrogate seems to be not enough to break tolerance to these self-antigens. Possible explanations according to the emerging paradigms (7, 8) could be: 1) "internal image" according to the pre-immune repertoire reference elicits a primary Ab response, 2) incomplete "interaction mimicry" might induce epitope-shifting affecting antibody specificity, 3) "structural restrictions" to idiotypic interactions might prevent a mature anti-anti-idiotypic response, 4) "clonal dominance" of natural autoantibodies might drive the inmune response hindering the clonal expansion of emergent specific clones. According to our results anti-Id MAb vaccines could be potentially useful for activating natural autoimmunity to cancer cells.

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