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Perspectives and Commentaries

Response to Monocolonal Antibodies in Melanoma: Specific or Non-specific?

ALEXANDER KNUTH

I. Medizinische Klinik und Poliklinik, Johannes Gutenberg-Universitaet, Mainz, West Germany

THE HYBRIDOMA technology to generate monoclonal antibodies added a new dimension to the definition of antigens on human malignant melanoma and other tumors. Of the large number of melanoma cell surface antigens defined to date none have actually been proven to be tumor specific [1, 2]. Some of these antigens are markers on melanocytes, expressed in different stages of their developmental pathway as well as on melanomas and other tumors of neuroectodermal origin [3]; by definition, they may therefore be considered differentiation antigens.

The murine monoclonal melanoma antibody R-24 detects an antigen identified as the disialoganglioside GD3 [4, 5]. When tested on viable cells and tissue sections by serological techniques, GD3-expression appears highly restricted to malignant melanomas and other tumors of neuroectodermal origin [6].

In vitro studies with this IgG3 antibody R-24 revealed some remarkable biological effects on cultured melanoma cells. R-24 alters titer-dependent the tumor cell morphology *in vitro*, exerts a growth inhibitory effect, and interferes with cell attachment. R-24 mediates human complement-dependent cytotoxicity and supports antibody-dependent cell mediated cytotoxicity with human effector cells [7–9].

Serological specificity and immunological properties of this unique antibody R-24 have prompted clinical trials with this agent in patients with malignant melanoma.

MONOCLONAL ANTIBODY EFFECTS ON HUMAN MELANOMA XENOGRAFTS

Monoclonal IgG3 antibodies recognizing the GD3 ganglioside antigen have recently been described by other investigators, too [10]. *In vitro* studies with these antibodies revealed ADCC-induction as well as complement-mediated cytotoxicity. Small xenografts of human malignant melanoma in nude mice were destroyed upon repeated intravenous administration of GD3 specific antibody 2B2.

GD2, another ganglioside expressed on tumors of neuroectodermal origin, has recently been defined by human monoclonal antibodies isolated from an Epstein–Barr-Virus-transformed human lymphoblastoid cell line [11]. This IgM antibody, when injected subcutaneously together with cultured human melanoma cells into nude mice markedly prolonged tumor free survival. Antibody and complement injected directly into tumor nodules suppressed tumor growth. Intraperitoneal antibody application, though, had no detectable anti-tumor effect.

Bumol *et al.* [12] described a monoclonal IgG antibody to a surface chondroitin sulfate proteoglycan on human melanoma cells mediating significant suppression of melanoma xenografts in nude mice. This effect was seen with antibody alone and with antibody coupled to diphtheria toxin A chain, suggesting host immune mechanisms like ADCC as the specific killing mechanism for the xenografts rather than the effect of the toxin conjugate, only.

MURINE MONOCLONAL ANTIBODIES IN HUMAN TRIALS FOR CANCER THERAPY

Several clinical phase I trials have been conducted over the past few years applying murine monoclonal antibodies alone or conjugated with radioisotopes to patients with various cancers [13, 14]. The most thoroughly investigated study has

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recently been published by Houghton *et al.* applying the same IgG3 antibody R-24 to melanoma patients [15], that had been used in Dippold's study.

Signs of inflammation around cutaneous tumor nodules after antibody infusions as first described by Dippold [16] have been observed regularly, beginning 2–4 hr after start of therapy. Symptoms varied from pruritus and urticaria around tumor sites or scars, where tumor once had been removed surgically, to more generalized reactions with mild wheezing in one patient. Nausea, vomiting and moderate temperature elevations were seen in two patients each. No other clinical side-effects were noticed.

Most remarkable, though, were the clinical antitumor effects in this trial. Three of twelve patients had major tumor regressions. Two patients had mixed responses, with progressive disease initially, but remarkable responses after treatment with dacarbazine following antibody infusion.

Serological studies showed human immunoglobulin against mouse Ig in all evaluable patients 15–40 days after antibody infusion. Tumor biopsies or aspirates after therapy showed continued expression of GD3 ganglioside antigen and tumor progression was not due to outgrowth of GD3 negative tumor. Inflammation around tumor nodules after antibody treatment correlated with dense mast cell infiltrates, degranulation of mast cells and complement deposition of components C3, C5, C9 and infiltrates of lymphocytes T3/8/Ia$^+$, findings not seen before therapy.

In other studies patients had been treated for leukemia or lymphoma with one complete remission in a B-cell lymphoma patient and minor responses in others [14, 17]. No side-effects of treatment generally were observed except for human anti-mouse Ig induction after monoclonal antibody infusion in some patients. Antibodies used in these studies were IgG1, IgG2a, IgG2b isotypes. In none of these studies have antibodies of IgG3 isotype been employed.

In another study cutaneous T-cell lymphoma had been treated by monoclonal IgG2a antibody infusion specific for the Leu-1 antigen on mature T-cells [18]. A partial remission of short duration was observed. In biopsies taken from lesions after antibody treatment a marked reduction of tumor cell infiltration without any detectable inflammatory cells was seen, much in contrast to the observations of Houghton *et al.* and Dippold. Human anti-mouse Ig also was not detectable in this patient. The model of cutaneous T-cell lymphoma probably most closely is comparable to the situation observed in malignant melanoma.

INFLAMMATION—SYMPTOM OF A SPECIFIC TUMOR RESPONSE?

Infusion of monoclonal mouse antibodies does not invariably induce an immune reaction in humans as can be concluded from clinical studies conducted to date. In cancer therapy with monoclonal antibodies questions of utmost importance are not only antibody specificity, but also antigen expression on target cells, quantitative differences in antigen expression and clonal heterogeneity of tumor and metastasis. Houghton as well as Dippold have described heterogeneity in GD3 antigen expression on malignant melanoma and metastasis [15, 4].

An antibody has to reach the target cell by means of sufficient vascularization. Excessively shed antigen may catch the antibody before the target cells are reached. Antigenic expression may be modulated by antibody–antigen contact or antibody may be engulfed into the target cell, a known phenomenon that may potentially be used for targeting tumor cells with coupled radioisotopes or cytotoxic drugs.

Previous attempts at influencing the course of malignant melanoma by non-specific immunotherapeutic approaches, applying bacterial vaccines, generally have failed. Local intralesional injections of those vaccines have induced inflammation and subsequent regression of nodules in malignant melanoma. Occasionally non-injected nodules in close proximity to injected nodules responded, too. Complete remissions of melanoma were rarely encountered and only in limited disease.

The pathophysiology underlying these observations is not fully understood, except for clear evidence of macrophage activation. Katano *et al.* [11] demonstrated tumor regressions after injecting monoclonal IgM antibody specific for GD2 into subcutaneous melanoma xenografts. When complement was injected simultaneously tumor regressions were observed but the histology of these regressions have not been investigated, yet.

Miller *et al.* [18] observed during treatment of cutaneous T-cell lymphoma with murine monoclonal antibody a lack of inflammatory responses in regressing lesions 4 weeks after antibody treatment. After 7 weeks, though, lymph nodes enlarged, showing bizarre mononuclear infiltrates with an intense inflammatory cell component and necrosis. Houghton *et al.* [15] observed urticaria and pruritus over tumor sites in skin as well as in scars where tumor had been removed previously but not in scars unrelated to tumor. Occasionally generalized urticaria was observed. Houghton's observations clearly were dose-dependent, namely

occurring only when more than 80 mg/m^2 of antibody had been infused, corresponding to findings as described by Dippold.

The extensive investigations of Houghton *et al.* in biopsies and aspirates from regressing tumor sites distinctively differ from the picture known from intralesional bacterial vaccines, in that the inflammatory infiltrate seen after a few weeks occurred in conjunction with a dense lymphocytic infiltrate of T3/8/Ia$^+$ cells. Until functional studies with isolated lymphocytes from lesions with these lymphocytic infiltrates have been done, their specific importance and impact remain unclear. It seems not unreasonable, though, to speculate over a likely sequence of events, underlining the specificity of tumor responses after monoclonal antibody infusion.

The unique functional properties of the IgG3 antibody R-24 as well as the nature of the target antigen GD3 may have contributed to the phenomena described after antibody infusion to melanoma patients. The immediate effects of the antibody on GD3-positive tumor cells known from *in vitro* studies changing morphology, cell surfce properties and potentially the antigenicity of the tumor cell for recognition by T-lymphocytes may provide an explanation for the T-lymphocytic infiltrate. Complement activation and ADCC induction are prominent properties of this unique antibody R-24 *in vitro*. These effects most likely are induced *in vivo* as well, explaining the complement deposition and also the inflammatory infiltrates as a consequence of the initiation of the complement cascade. Initiation of these events specifically over tumor nodules but not tumor unrelated sites after systemic antibody application argues for the specific nature of the inflammatory and tumor response.

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