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Review

Monoclonal Antibodies as Agonists: An Expanded Role for Their Use in Cancer Therapy[1]

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Introduction

The development of technologies to generate MAbs[3] (1) created considerable excitement in oncology because of their potential use for tumor therapy. The initial rationale was to harness the exquisite specificity of antibodies to bind to tumor-specific antigens and, thereby, to kill tumor cells by means of conventional effector mechanisms that have been perfected during the long evolution of the mammalian immune system, *e.g.*, opsonization, ADCC, or complement-mediated lysis (2, 3). When MAbs were evaluated for cross-reactivity with normal tissues, however, it became apparent that the majority of tumor-associated antigens were not tumor specific (4), thus creating an apparent obstacle to the above strategy. However, the density of such antigens (*e.g.*, interleukin receptors and carbohydrate moieties peculiar to tumors) was often increased on cells from particular tumors (5–7), thereby providing an operational window of specificity. In addition, lineage-specific antigens can serve as targets, provided that stem cells in the lineage are antigen-negative and, hence, able to reconstitute the cellular compartment or the tissue involved. Subsequent to selection for MAbs of suitable specificity, the isotypes of such MAbs were then selected to maximize effector functions such as ADCC (8).

Despite this initial intellectual appeal, the general therapeutic efficacy of tumor-reactive MAbs has been disappointing. In particular, the results of clinical studies in patients with solid tumors showed little efficacy (9–13), except in the setting of minimal disease (14). This relates in part to the fact that patients in Phase I trials usually have large tumors with poor access to circulating MAb. In addition, the above criteria for selecting MAbs may not have been optimal, as will be discussed in this article.

In contrast to results with carcinomas, significant success has been reported in treating NHL and T-cell leukemias with tumor-reactive antibodies. Levy and Miller (15) and Hamblin *et al.* (16) have used anti-idiotope MAbs to treat NHL and chronic lymphocytic leukemia, respectively. In the majority of cases of NHL, there have been partial or complete remissions using single anti-idiotopic antibodies. Relapses frequently indicated the emergence of idiotope-negative variants (17, 18). Dyer *et al.* (2) have also obtained impressive anti-tumor effects in NHL with anti-CD52. Finally, anti-CD25 has shown some efficacy in the treatment of human T-cell lymphotrophic virus I-induced adult T-cell leukemia (19). These results have demonstrated both the effectiveness of some antibodies in eliminating neoplastic B- and T-cells and the problems associated with the generation of antigen-negative variants.

Despite the results of clinical studies in patients with epithelial tumors and the above mentioned limitations of MAb therapy, we believe that the potential of MAbs as therapeutic agents has not been thoroughly explored. The purpose of this review is to reevaluate the prevalent concept that the major antitumor effects of these antibodies are due to the harnessing of conventional effector mechanisms of the host. We will review evidence supporting an alternative interpretation, *i.e.*, that antibodies directed against cell surface molecules on many types of tumor cells can act as ligands, resulting in powerful antitumor effects mediated by signal transduction. If MAbs are selected by virtue of this characteristic, they may serve as important adjuncts to conventional chemotherapy. We will use B- and T-cell tumors as the major example but will also discuss breast carcinoma.

Role of Effector Functions of MAbs

There is considerable experimental and some clinical evidence to indicate that the effector functions of MAbs can play a major role in tumor immunity. This issue has been studied by two approaches: (*a*) the use of class switch variants of tumor-reactive antibodies in humans, human tumor/nude mouse models, or murine tumor models as well as in *in vitro* cytotoxic assays; and (*b*) analysis of the effects of different human immunoglobulin isotypes involving large panels of tumor-reactive antibodies, both for their experimental *in vivo* antitumor effects and their cytotoxic effects *in vitro*. Both approaches have led to the same conclusion, *i.e.*, that *in vivo*, opsonization and ADCC can play critical roles in antitumor activity (20, 21) and that murine IgG2a (8, 22, 23) is by far the most effective isotype. The effectiveness of IgG2a correlates with its capacity to interact with host effector cells. Similar results were obtained in mice using switch variants of tumor-reactive antibodies (24), *i.e.*, IgG2a was the most effective. However, all isotypes showed some antitumor activity. Although *in vitro* assays indicated that the ability of MAbs to bind complement was important (25), no evidence was provided to indicate that this effector function operated *in vivo*. Indeed, complement depletion of Nude mice bearing human xenografts did not affect the antitumor function of IgG2a MAbs (8). Macrophages were thought to play an important role in the antitumor effects of IgG2a because agents that damaged macrophages abolished the tumoricidal effect of the MAb (8). In the studies by Dyer *et al.* (2), rat or humanized MAb specific to CD52 was used to treat NHL. By studying a switch variant of the rat MAb, they showed that IgG2b was far superior to IgG2a in treating NHL in a study in which two patients were each treated with one of the antibody isotypes. Both MAbs were able to remove peripheral neoplastic lymphocytes, but only the IgG2b antibody produced a long-lasting depletion of lymphocytes from blood and tissues, whereas the IgG2a tumor cells rebounded rapidly. Additional clinical observations support the role of IgG2b in killing lymphoma cells (2). In extensive experimental studies, rat IgG2b was more effective than

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[3] The abbreviations used are: MAb, monoclonal antibody; ADCC, antibody-dependent cellular cytotoxicity; NHL, non-Hodgkin's lymphoma; CCA, cell cycle arrest; DLC, dormant lymphoma cell; IT, immunotoxin; *erb*B-2R, *erb*B-2 receptor; IL, interleukin.

other isotypes in killing lymphoid cells *in vivo* or mediating cellular cytotoxicity (ADCC) *in vitro* (20). With regard to human MAbs, IgG1 and IgG3 are most effective in inducing ADCC (26), but IgG4 from some patients can demonstrate such function *in vitro* (27).

In summary, there is evidence that antibody-induced opsonization and cellular cytotoxicity can induce antitumor effects, but their importance in antibody-induced tumor immunity is not clear. Certain immunoglobulin isotypes, depending upon the species of origin and the host, appear to be most efficacious with regard to these effector functions. There are additional issues that remain unsolved. Thus, the relative contributions of ADCC, opsonization, and cytostasis to these antitumor effects are not known. Importantly, there appears to be variation among individuals in regards to the role that the different isotypes can play in MAb-induced tumor immunity (27). Indeed, MAbs display isotypic polymorphisms in the human that may account for these differences.[4] If so, these immunoglobulin isotypes with minimal amino acid differences may provide critical clues to the structural motifs involved in ADCC and other effector functions.

Signaling Functions of Antibodies

The Immunoglobulin Receptor Complex on B Cells (Fig. 1). B cells usually express cell surface IgM and IgD with identical variable regions (28). By themselves, the heavy chains of these molecules are incapable of signaling because of very short cytoplasmic tails (29, 30). However, each H chain is associated with an Igα and Igβ (or Igγ) molecule (31, 32) that contains structural motifs that can bind members of the *src*-family protein tyrosine kinases, *e.g.*, Lyn (33), Fyn, Blk (34–36), Lck (36), and SYK (37–39). After cross-linking IgM or IgD on mature B-cells, protein tyrosine kinases become enzymatically active, resulting in the phosphorylation of Igα and Igβ, the kinases themselves, and several other intracellular protein targets (32, 40–43). This initiates a cascade of biochemical events along two major pathways, resulting in cellular proliferation and/or differentiation (41, 44–46). These include phosphoinositide metabolism with activation of PLCγ and generation of Ins P3 and diacylglycerol that result in elevations in intracellular Ca^{2+} and activation of protein kinase C (47). Protein kinase C (a serine/threonine kinase) then regulates transcriptional activity of the AP-1 complex (48). A second pathway involves activation of Ras and another series of intermediate messengers (GAP, Grb2, Raf, MAPKK, and MAPK; Refs. 49–51), resulting in phosphorylation of c-Jun, a transcriptional regulator (52). Other possible pathways include phosphatidylinositol 3-kinase (42, 53–55), which may act downstream on NF-κB (56) and the recently described Jak proteins involved in signaling by cytokine receptors (57).

In summary, the major pattern for B-cell receptor signaling is that cross-linking of membrane immunoglobulin stimulates activation of protein tyrosine kinases as proximal events which, via a series of intermediates, stimulate serine/threonine kinases, which in turn regulate gene transcription (distal events). However, the precise sequence of reactivities, the relationships between the components of this complex signaling cascade, and their regulation are not yet well defined.

The Ig signaling complex is not limited to membrane immunoglobulin, Igα, and Igβ (Fig. 1). There are additional molecules on the B-cell surface, *e.g.*, CD19 (58–60), CD20 (60–62), CD21 (58), CD22 (63), CD24 (44), CD32 (44), CD45 (64), leu13 (65), and CD81 (TAPA-1; Refs. 44, 46, 66, and 67), which can interact with or affect signaling by the immunoglobulin complex. Interactions with these molecules (with the exception of CD32; Refs. 68 and 69) usually enhance the immunoglobulin-mediated signals that lead to the activation of normal B-cells. In addition, antibodies directed against these molecules can induce signals in normal B-cells in the absence of IgM cross-linking (66, 70–72). The outcome of signaling after the cross-linking of surface immunoglobulin or the other molecules in the receptor complex depends upon the stage of differentiation of the B-lymphocyte. Mature B-cells proliferate and differentiate into immunoglobulin-secreting and memory cells; immature B-cells and certain B-cell lymphomas undergo CCA or apoptosis (73–79), as will be discussed.

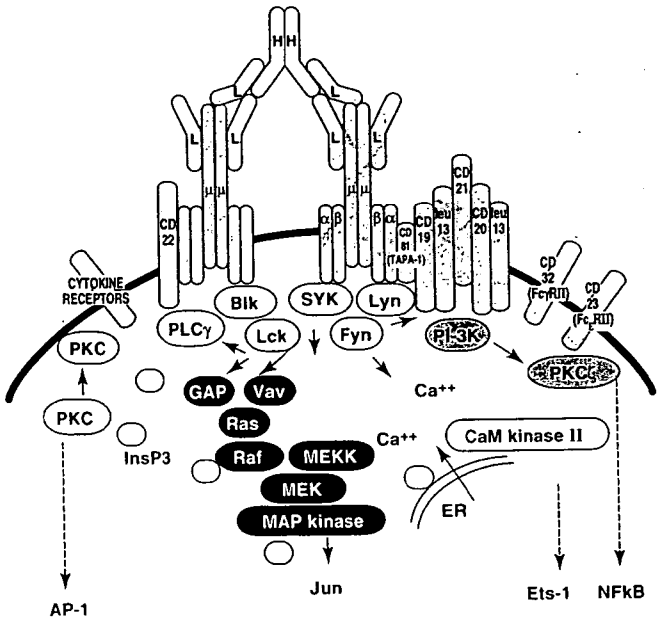

Fig. 1. Model of negative signaling in B-cells by cross-linking IgM [adapted from Cambier *et al.* (157) and Fearon *et al.* (158)]. This model illustrates some of the components of the membrane IgM signaling cascade that may be involved in the induction of cell cycle arrest and/or apoptosis. The model shows surface IgM cross-linked by an IgG anti-μ chain antibody. The Igα and Igβ (or Igγ) chains associate with the μ chains. For simplicity, a number of molecules have been depicted as part of the Ig signaling complex, although in some cases, the evidence is incomplete. The small number of *arrows versus* the large number of intracellular second messengers reflects a lack of understanding of the precise interactions and sequence of events that take place after membrane IgM is cross-linked.

Negative Signaling in Neoplastic B-Cells. The earliest studies *in vivo* indicating that antibodies against surface immunoglobulins on B-cells had antitumor effects were those of Lynch *et al.* (80) who showed that immunization of mice with myeloma proteins induced an antitumor response following challenge with myeloma cells. This response was attributed to T-cells, which were thought to suppress tumor cell growth. Krolick *et al.* (81) described the induction of a dormant tumor state in mice bearing an aggressive lymphoma (BCL_1) if mice with advanced tumor were treated with irradiation, splenectomy, and an anti-Id or anti-δ immunotoxin. Although treated mice appeared clinically normal for 1 year following treatment, after sacrifice, many of their tissues were able to transfer tumor to naive recipients. The antitumor effect in the donor mice was presumed to be an anti-Id response to the initially massive tumor. In this regard, George *et al.* (82) and Stevenson *et al.* (83) showed that immunization with the monoclonal BCL_1 immunoglobulin induced an anti-idiotypic response that led to a state of dormancy in mice challenged with the BCL_1 tumor cells. Injection of anti-idiotype-containing serum into naive mice that were challenged with BCL_1 tumor cells resulted in a state of dormancy in some of the recipients (82, 83). We have used this model to demonstrate that the major outcome of such immunization in over 500 mice is a dormant state and that such mice carry DLCs for as long as 2 years (79). Dormancy was also induced in the

[4] H. Waldmann, personal communication.

vast majority of SCID mice receiving antibodies directed against epitopes on the immunoglobulin molecule of the BCL_1 tumor cells, proving that antibody can induce dormancy (79). Although insufficient by themselves, Id-immune T-cells could enhance the induction and the duration of dormancy induced by antibody.[5] Thus, just as in humans with B-cell lymphoma (15, 16), anti-Id can be highly effective as an antitumor agent in mice with the BCL_1 lymphoma. Multiparameter flow cytometry was used to isolate and characterize the DLCs. The DLCs were physiologically different from BCL_1 cells growing in naive mice; they were smaller in size, appeared less "malignant" morphologically, had a different profile of oncogene expression and a proportion of these cells were in CCA (79). Since the size of the population of DLC was stable for many months, it was presumed that cell death was balancing residual cell replication.

There is a large body of *in vitro* evidence to support the notion that the *in vivo* results may depend heavily on signal transduction mechanisms. Indeed, it has been shown that there is a correlation between clinical responses to anti-Id and the capacity of anti-Id to induce phosphorylation of proteins in tumor cells freshly prepared from patients with NHL (84). It is known that the cross-linking of IgM on many but not all murine lymphoma cells can result in growth arrest in G_1 followed by apoptosis (40, 41, 46, 73, 74, 76, 77, 85–87). Both phosphorylation of tyrosine residues on the src-like kinases and phosphoinositide hydrolysis (47) are important components. It has been postulated that the above events reflect a physiological mechanism underlying tolerance to self by which normal immature B-cells undergo clonal anergy or deletion following interaction with self antigens (88, 89). The generation of CCA can also be induced in mature B-cells when surface IgM is cross-linked in the absence of a T-cell signal (89). However, in some B-lymphomas, cross-linking does not appear to signal negatively (90, 91), either because the cells represent a more advanced stage of maturation or signaling can no longer override the uncontrolled growth signals inherent in these particular tumor cells.

Other Molecules in the Ig-Signaling Complex. CD19 is part of the multimolecular immunoglobulin receptor complex on the B-cell and can associate with membrane immunoglobulin, leu13, CD81, and CD21 (58, 66). Although CD19 associates with membrane immunoglobulin, signaling through CD19 is apparently distinct, since it displays differences in Ca^{2+} flux, PIP_2 turnover kinetics, phosphoprotein patterns, involvement of protein kinase C (92), and apoptotic responses (71). The importance of CD19 signaling in the antitumor activity of anti-CD19 antibody was suggested by experimental studies on the efficacy of an CD19-ricin A chain IT in SCID mice with Daudi cell xenografts (93). It was demonstrated that the anti-CD19 antibody (HD37) alone was as effective as its respective IT in inhibiting growth of several human B-lymphomas in SCID mice. The inhibition was immunologically specific because isotype-matched control IgG_1 or an anti-CD22 (RFB4) antibody alone (although potent as an IT) had no antitumor activity. When HD37 was administered with the RFB4 IT, the combination cured mice of minimal disease; neither IT nor antibody alone (even at high doses) was curative (94). More importantly, the $F(ab')_2$ fragments of HD37 were as effective as the intact antibody when doses were adjusted for the 10-fold longer half-life of the latter in SCID mice (71). These experiments indicate that the antitumor effect of HD37 is not mediated by conventional effector mechanisms in the host and, therefore, suggests that the beneficial results involve signal transduction. This interpretation is fully supported by *in vitro* studies, which demonstrate that both intact anti-CD19 antibody and its $F(ab')_2$ fragments induce CCA in several human lymphoma cell lines (71).

Using a panel of anti-CD19 MAbs, it was found that their ability to induce CCA depended both on their affinity and the epitope on the CD19 molecule which they recognized (71). This is an important point operationally because it means that, to determine signaling potential, a panel of MAbs against a given molecule should be studied to have a reasonable chance of detecting one that reacts with the appropriate epitope and has the necessary affinity to deliver a signal. In contrast to anti-CD19, treatment of Daudi cells *in vitro* with anti-μ induced both CCA and apoptosis (78).

Taken together with other findings, these observations raise the possibility that CCA and apoptosis may involve two distinct signaling pathways in neoplastic B-cells. In this regard, anti-sense lyn experiments in Daudi cells treated with anti-CD19 or anti-μ were carried out to determine if src-family kinases are critical for inducing CCA and/or apoptosis. The selection of anti-sense-lyn was based on previous findings that lyn is associated with CD19 (59) as well as membrane immunoglobulin (33). Pretreatment with anti-sense lyn before the addition of anti-CD19 or anti-μ completely prevented the induction of CCA by the cross-linking of CD19 or IgM. In contrast, induction of apoptosis by anti-μ was not inhibited (78). These results are similar to those obtained with a cell line (3B3) derived from the mouse BCL_1 tumor cells (78). These results suggest that there are two signaling pathways, a CCA-pathway that is lyn-dependent and an apoptosis pathway that is lyn-independent. Anti-sense studies by Yao and Scott (91) suggest that Blk may be critical for inducing apoptosis.

Additional evidence suggests but does not prove that other B-cell-reactive antibodies can deliver negative signals to tumor cells. In the report of a recent clinical trial conducted by Kaminski *et al.* (95), anti-CD20, coupled to well-tolerated amounts of ^{131}I, was administered to patients with NHL. Durable remissions were achieved in a significant number of patients with few side effects, and the marrow was unaffected. In a human SCID/B-lymphoma model, the cold anti-CD20 MAb was a more potent antitumor agent than its ^{131}I-conjugate (96). Hence, the antibody itself may have played a major role in the antitumor activity observed clinically, although the mechanisms are unclear (97). The above interpretation is consistent with an earlier study which showed that unlabeled anti-CD20 administered to patients with NHL induced dose-dependent tumor regressions with a partial response (over 50% tumor reduction) in a patient receiving the largest amount of antibody (1 g; Ref. 98). Anti-TAPA-1 (CD81) can also induce a reversible antiproliferative effect on a human lymphoma cell line (66, 99). There is also evidence to suggest that anti-CD21, anti-CD23, and anti-CD24 can down-regulate the growth of Epstein-Barr virus-positive tumors in SCID mice (100) and humans (101). As mentioned before, these molecules are part of the immunoglobulin signaling complex (44, 46, 58–62, 67, 102–106); therefore, it is possible that their antitumor effects result from signal transduction. To distinguish between effector function and signaling, however, experiments comparing IgG antibody and its $F(ab')_2$ fragments *in vivo* will be necessary.

Effector Functions *versus* Signaling

How can one reconcile the data indicating an important role in tumor immunity for effector functions of MAb with the data indicating that agonist activity is critical and that effector functions may play a minor role? In the past, MAbs were selected as antitumor reagents by virtue of their specificity for the tumor and then, secondarily, for their effector function. The effectiveness of a particular antibody was entirely dependent on its ability to recruit conventional effector function(s), unless it coincidentally possessed negative signaling capacity. It is not surprising, therefore, that the agonist function of antibodies was inadvertently obscured by this biased process of selection. Only when MAbs are selected for negative signaling functions will it be

[5] E. Racila, R. Scheuermann, L. Picker, E. Yefenof, T. Tucker, W. Chang, R. Marches, N. Street, E. S. Vitetta, and J. W. Uhr, submitted for publication, 1994.

possible to assess whether or not there is an additive role for MAbs acting through effector functions. Indeed, it is likely that this will be the case. If so, a signaling antibody could be altered by genetic engineering to introduce the desired Fc portion. Moreover, a nonsignaling antibody could be used on the basis of its effector function, and another antibody with a different specificity could be used simultaneously for its signaling ability. New information regarding signaling will facilitate the design of experiments to address the relative contributions of these two antitumor effects of antibody and to explore various regimens to optimize their therapeutic use.

Anti-*erb*B-2R Signaling of Carcinoma

Negative signaling of B-cell tumors by cross-linking IgM or other molecules on the cell surface could be considered a phenomenon unique to lymphocytes since the normal cellular counterpart, an immature B-cell, is destined to become anergic or deleted following interaction of its membrane immunoglobulin with self antigens as a means of establishing self tolerance. The question then arises as to whether negative signaling from MAbs can occur in nonlymphoid neoplasms. In this regard, there is considerable evidence that epidermal growth factor receptor (107) and *erb*B-2R (also known as $p185^{HERR2}$ oncoprotein) on breast, ovarian, and several other types of carcinomas (108, 109) can also function as a suitable target for negative signaling by MAbs (109–113). For simplicity, we will discuss only the *erb*B-2R. *erb*B-2R is a member of the epidermal growth factor receptor family (114) and is presumed to act as a signaling receptor for a yet-to-be-identified ligand concerned with regulation of growth and differentiation of breast cells and other cell types. Overexpression of *erb*B-2R on breast cancer cells is associated with a poor prognosis (115–122). If a MAb with sufficient affinity against a particular epitope on *erb*B-2R is added to *erb*B-2R-overexpressing breast or ovarian carcinoma cells, a strong antiproliferative effect can be induced (109, 111, 123, 124). One example is the MAb anti-*erb*B-2R 4D5, which in ng concentrations can inhibit proliferation of breast cancer cells that overexpress *erb*B-2R (108, 123) and is presently being evaluated in clinical trials. It is presumed to act via signal transduction because of its antiproliferative effect *in vitro* and the accumulating body of evidence that cross-linking of *erb*B-2R induces a series of biochemical changes associated with a signaling cascade (125). We have recently shown that 4D5 induces both CCA and cell death in *erb*B-2R overexpressing breast cancer cells and that these effects require functional tyrosine kinases.[6] Thus, as with B-lymphoma cells, in breast cancer cells, there appear to be two pathways, one for CCA and another for induction of cell death.

It is not surprising that nonlymphocytic neoplastic cells can be signaled by cross-linking particular surface molecules since these may play major roles in the regulation of cellular growth and differentiation. This does not imply that all tumor cells will be susceptible to such regulation. However, we would speculate that a proportion of tumors of many cell lineages express surface molecules which, when extensively cross-linked, may deliver sufficiently strong signals to override the malignant phenotype and induce either CCA or death.

Additive Effects

It is of particular interest that, *in vitro*, simultaneous addition of anti-CD19 (which by itself induces CCA) and anti-μ (which can induce both CCA and apoptosis) to human B-lymphoma cells results in an increase in the proportion of apoptotic cells.[7] Besides enhancing negative signaling, an additional benefit to using two (or more) antibodies specific for molecules on the same tumor cell is the inhibition of emergence of antigen- or epitope-negative variants. Thus, Levy and Miller (15) and Kwak *et al.* (126) have combined two or more anti-idiotopes to lessen the possibility that idiotope-negative NHL cells will escape inhibition.

Similar results have been obtained with anti-*erb*B-2R antibodies. There are reports of additive antitumor effects when two anti-*erb*B-2R MAbs are used simultaneously *in vitro* (127, 128) or in nude mice (112, 127). These experiments, therefore, support the strategy of searching for combinations of MAbs that display such additive effects. There are several types of combinations to consider, none of which are mutually exclusive: (*a*) a MAb directed against a surface molecule that induces CCA and another directed against a different surface molecule that induces cell death; (*b*) MAbs that bind to two different molecules, resulting in signaling *via* the same intracellular pathway; and (*c*) MAbs that bind to different epitopes on the same molecule. Information concerning these combinations will be critical in developing an *in vitro* paradigm for predicting the efficacy of antitumor MAbs *in vivo*.

Are the Negative Signals Physiological?

It seems reasonable to assume that cross-linking of receptors by MAbs that signal CCA and apoptosis are imitating the signals induced by physiological agonists. For example, tolerance induction to self antigens on immature B-cells requires signaling through the immunoglobulin receptor complex as discussed above. However, there are theoretical considerations as well as experimental data that suggest a more complicated interpretation. Thus, the interaction of a physiological ligand with a small percentage of receptors on a cell and cross-linking a proportion of them should be sufficient to deliver a signal. Nature would be expected to provide a considerable excess of such receptors to ensure signaling when required. In contrast, if all the receptors are cross-linked and, indeed, clustered into large aggregates by MAbs, a different signal, quantitatively or qualitatively, might be expected. These theoretical considerations are indirectly supported by a number of studies. Thus, optimal negative signaling of B-cell tumors requires concentrations of antibody that exceed the number of IgM molecules on the cell surface, indicating that both saturation of surface IgM and cross-linking of newly expressed IgM during the incubation period of one or more days is required for a maximum effect (129–131). As previously mentioned, the same is true for studies of cells overexpressing *erb*B-2R in which increased cross-linking increases the negative signaling (112, 127) and the proportion of cells that die.[6] In addition, the frequency and magnitude of elevations of intracellular calcium ions change markedly as concentrations of ligand (132) (including anti-μ) (133) are increased from physiological to pharmacological levels. It is possible, therefore, that the antitumor effects induced by MAbs acting as agonists may be different in intensity and/or quality from those induced by the physiological ligands at their usual concentrations.[8] Indeed, such abnormal signaling may by responsible for inducing apoptosis. In this regard, it has recently been shown that anti-μ and anti-δ bound to plastic can induce apoptosis in normal B-cells (134).

[6] R. Marches, R. H. Scheuermann, L. Picker, T. T. Tucker, E. Racila, N. E. Street, G. Shen, J. Li, B. Wei, A. Ilgen, E. S. Vitetta, and J. W. Uhr, submitted for publication.

[7] E. Racila, R. Scheuermann, L. Picker, T. Tucker, R. Marches, N. Street, E. S. Vitetta, and J. W. Uhr, unpublished results.

[8] It is possible, that at particular stages of development, ligand concentrations are markedly increased in order to induce apoptosis in a particular cell lineage and, in that sense, are physiological.

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