

Immunity to p53 Induced by an Idiotypic Network of Anti-p53 Antibodies: Generation of Sequence-specific Anti-DNA Antibodies and Protection from Tumor Metastasis¹

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ABSTRACT

The general overexpression of p53 by different types of tumor cells suggests that p53 immunity might be generally useful for tumor immunotherapy. We describe here the induction of immunity to p53 and resistance to tumor metastasis using an idiotypic network. Mice were immunized with domain-specific anti-p53 monoclonal antibodies (Ab1): PAb-248 directed to the N-terminus; PAb-246 directed to the specific DNA-binding region; or PAb-240 directed to a mutant p53 that does not bind specific DNA. Immunized mice responded by making anti-idiotypic antibodies (Ab2) specific for the Ab1 inducer. Ab1 PAb-246 induced Ab2 that, like p53 itself, could bind the specific DNA oligonucleotide sequence of the p53 responsive element. Mice immunized with Ab1 PAb-240 or PAb-246 spontaneously made Ab3 anti-p53 antibodies that reflected the specificity of their Ab1 inducers: Ab1 PAb-246 induced Ab3 specific for wild-type p53; PAb-240 induced Ab3 specific for mutant p53. Ab1 PAb-248 induced only Ab2. The spontaneously arising Ab3 were of T cell-dependent IgG isotypes. Peptides from the complementarity determining regions of the Ab1 antibodies PAb-240 and PAb-246 could also induce Ab3 anti-p53. Finally, mice that produced Ab3 anti-p53 acquired resistance to tumor metastases. Therefore, an anti-idiotypic network built around certain domains of p53 seems to be programmed within the immune system, specific Ab2 antibodies can mimic the DNA binding domain of p53, and Ab3 network immunity to p53 can be associated with resistance to tumor cells.

INTRODUCTION

Inactivation of p53 plays a central role in the development of tumors because p53 normally functions to inhibit the growth of aberrant cells (1). Functional p53 is believed to sense DNA damage (2) and activate either DNA repair (3) or apoptosis of the aberrant cell (4). Loss of normal p53 activity in a tumor cell can occur through mutation of the *p53* gene (5) or through mechanisms that functionally inactivate the unmutated, wild-type p53 protein (6). Irrespective of the molecular basis, inactivation of p53 is associated in most cancer cells with overexpression of the p53 protein. The fact that, irrespective of their origin, a majority of cancers accumulate p53 suggests that p53 immunity might be generally useful for cancer immunotherapy. Indeed, it was shown that peptides derived from mutant, as well as from wild-type p53, can bind to MHC molecules and be recognized by CTLs (7). Anti-p53 CTLs were shown to lyse various tumor cells (8) and to protect mice from tumor challenge (9). Presently, p53-specific

immune responses are usually induced by immunization with whole p53 or with p53 peptides. To explore another approach to p53 immunity, we have undertaken idiotypic immunization.

In his idiotypic network theory, Jerne (10) suggested that a given antibody (Ab1) can react not only with an epitope on its target antigen, but with antigen binding sites of other, Ab2 antibodies. Since an Ab1 antibody recognizes both the antigen epitope and the Ab2 antibody, the Ab2 antibody might mimic the antigen epitope structurally (11). Continuing the chain reaction, Ab2 antibodies also might be recognized by a set of antibodies, Ab3, some of which could have the same antigen specificity as the Ab1 antibody. According to Jerne's original formulation, an idiotypic network could be triggered equally well by any Ab1 without bias for particular specificities. Be that as it may, it has been shown that some Ab2 antibodies can replace tumor antigens as immunogens and induce immune responses to tumor-associated antigens (12). T cells have been found to be involved in such idiotypic networks (13).

In a preliminary study, we found that idiotypic immunization of BALB/c mice with a monoclonal Ab1 antibody specific for mutant p53, PAb-240 (14), induced resistance to the Meth A tumor bearing a p53 mutation (15). In the present study, we extended our original observation by immunizing BALB/c or C57BL/6 mice with different Ab1 anti-p53 antibodies and characterized the underlying idiotypic immune responses. The C57BL/6 mice were challenged with the spontaneously metastasizing 3LL tumor. The three monoclonal Ab1 antibodies to different domains of p53 were: (a) PAb-246, which recognizes the specific DNA binding domain in the core of p53 in its native conformation (16, 17); (b) PAb-240, which recognizes the core of p53 in a mutant conformation (14); and (c) PAb-248, which binds to a domain located at the N-terminus of the p53 molecule (16) and recognizes both mutant and wild-type p53. Using these three Ab1 antibodies and linear peptides derived from their CDRs,⁴ we have found: (a) that the anti-p53 idiotypic network is biased to only some Ab1 specificities; (b) that the Ab2 antibodies induced by PAb-246 can recognize the p53-specific DNA sequence; (c) that Ab3 antibodies can preserve the p53 domain specificity of the Ab1; and (d) that activation of the anti-p53 idiotypic network can lead to inhibition of lung metastases.

MATERIALS AND METHODS

Mice. Female BALB/c and C57BL/6 mice were obtained from the animal breeding facilities at the Weizmann Institute of Science and used at the age of 8–10 weeks.

Antibodies and Immunizations. Immunization was done with the anti-p53 antibodies PAb-240 (16), PAb-246 (16, 17), or PAb-248 (16) purified from ascitic fluid by Protein A affinity chromatography (Sigma Chemical Co., Rehovot, Israel). As an isotype-matched (IgG1) control antibody, R73 was used, which is specific for the rat T-cell receptor (18). Mice received injections

Received 6/22/98; accepted 10/5/98.

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¹ Supported by grants from the Minerva Foundation and the Human Frontier Science Program Organization (HFSP). I. R. C. is the incumbent of the Mauerberger Chair of Immunology and the director of the Robert Koch-Minerva Center for Research in Autoimmune Diseases.

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⁴ The abbreviations used are: CDR, complementarity determining region; VH, variable region of the antibody heavy chain; VL, variable region of the antibody light chain.

in the hind footpads with 20 μg of antibody emulsified in complete Freund's adjuvant. After 3 weeks, the mice were boosted s.c. in the flank with 20 μg of the same antibodies in incomplete Freund's adjuvant. Sera were obtained 10 days after the boost.

CDR Peptides. The sequences of the heavy and light chains of Ab1 anti-p53 monoclonal antibodies were derived as described (19). The CDR peptides were prepared using an automated synthesizer (Abimed model AMS 422; Langenfeld, Germany) according to the company's protocol for N-fluorenylmethoxycarbonyl synthesis. Peptide purity was tested by analytical reverse phase high-performance liquid chromatography and mass spectroscopic analysis.

Preparation of Recombinant p53. *Escherichia coli* BL21 (DE3) cells were transformed with the T7 expression vector containing mouse p53 cDNA (20). Purification of p53 was done as described (21).

ELISA. ELISA assays were done in 96-well Maxisorp plates (Nunc, Roskilde, Denmark). Ab2 anti-idiotypic antibodies were detected by coating plates with 10 $\mu\text{g}/\text{well}$ of (Fab)₂ fragments of PAb-240 or of PAb-248 in PBS. The (Fab)₂ fragments were prepared as described (22). Antibodies to p53 were detected by coating the wells with recombinant p53 at 10 $\mu\text{g}/\text{ml}$ in PBS. After washing and blocking with 1% BSA in PBS for 1 h at 37°C, test sera (0.1 ml/well) were added for 1 h at 37°C followed by a 1-h incubation with goat antimouse IgG Fc specific, or IgG-isotype-specific secondary antibodies conjugated to alkaline phosphatase, diluted 1:5000 (Jackson, Philadelphia, PA). After washing, bound antibodies were detected by the addition of a substrate solution containing 0.6 mg/ml p-nitrophenylphosphate (Sigma Chemical Co) in diethanolamine-H₂O (pH 9.8) and read at 405 nm. The antibody reactivities, shown as the optical density, were produced by the test sera at a dilution of 1:100. The *dots* in Figs. 1, 3, and 5 represent individual mice, and the *bars* represent the median value of the group. Each group in Figs. 1 and 3 consisted at least of nine mice; each group in Fig. 5 consisted of six mice. Note that some of the figures may actually show fewer dots, due to the superposition of mice with identical values.

Band Shift Assay. We used a band shift assay to detect antibodies to the p53-specific nucleotide sequence. The p53-responsive element consensus sequence oligonucleotide TCGAGAGGCATGTCTAGGCATGTCTC (23) was synthesized, prepared in a double-stranded form, and end labeled. We also used a substituted oligonucleotide sequence, TTGAGGCAAGGCAAGGCAAGGCCAC (substitutions are underlined; Ref. 23). DNA (10–20 fmol) was mixed with either 4 μl of test sera, or with 1 μg of recombinant p53, activated by PAb-421 (21). Poly dI-c2 (2 μl) and a half reaction volume (8 μl) of buffer (25 mM Tris-HCl, 100 mM KCl, 6.25 mM MgCl₂, 0.5 mM EDTA, 1 mM DTT, and 10% glycerol) were added. The reagents were mixed, incubated for 15 min on ice, and incubated for 15 min at room temperature. The reaction products were separated by a 4% PAGE for 3 hours in a 0.4% TBE running buffer. As indicated, 2 μl of PAb-246 ascitic fluid were added.

Indirect Immunoprecipitation. Meth A cells were metabolically labeled for 1 hr at 37°C in methionine-free DMEM supplemented with 10% heat-inactivated FCS and 0.125 mCi/ml of [³⁵S]-methionine (Amersham Corp., Little Chalfont, United Kingdom). The cells were lysed in a buffer containing 10 mM phosphate buffer (pH 7.5), 100 mM NaCl, 1% Triton, 0.5% sodium deoxycholate, and 0.1% SDS and precleared on 50% protein A-Sepharose CL-4B (Sigma Chemical Co). The cell lysates were incubated overnight at 4°C with monoclonal anti-p53 antibodies or with test sera. Immune complexes were precipitated with protein A-Sepharose CL-4B for 2 hr at 4°C, followed by 3 washes with PBS. The immunoprecipitates were separated on 10% SDS-PAGE gels and detected by autoradiography.

3LL Lung Carcinoma. The D122 highly metastatic variant of the 3LL lung carcinoma cell line (24) was used for *in vivo* tumor challenge experiments. C57BL/6 mice received injections of 2×10^5 3LL cells, a gift of Prof. Lea Eisenbach (Weizmann Institute), into one hind footpad. Local tumor growth was measured using a caliper, and the tumors were excised when they reached 8 mm. The mice were sacrificed 21 days after tumor removal, and their lungs were weighed as a quantitative measure of the metastatic load (25). Lung weights of ≥ 200 mg indicate the presence of metastases in individual mice (*dots*). The *bars* illustrate the median lung weight of the group.

Statistics. The differences between experimental groups were tested for significance with the nonparametric Mann/Whitney *U* test.

RESULTS

Ab1 Antibodies Induce Ab2 Antibodies. BALB/c or C57BL/6 mice were immunized with Ab1 anti-p53 antibodies PAb-240, PAb-246, or PAb-248, all of the IgG1 isotype. We used R73, which is specific for the rat T-cell receptor (18), as an isotype-matched control antibody. Sera from the immunized mice were assayed for Ab2 reactivity to the (Fab)₂ fragment of PAb-240, PAb-246, or PAb-248. Fig. 1 shows that both BALB/c and C57BL/6 mice made specific anti-idiotypic Ab2 antibodies. The mice immunized with PAb-240 made Ab2 antibodies that specifically reacted with PAb-240 (Fig. 1A), and the mice immunized with PAb-248 made Ab2 that specifically recognized PAb-246 (Fig. 1B). Immunization with PAb-248 also induced specific Ab2 (Fig. 1C).

Ab2 Induced by PAb-246 Bind a Specific DNA Sequence. The DNA binding domain of p53 that is recognized by PAb-246 can interact specifically with a DNA sequence in p53-reactive promoters. The p53-specific DNA sequence consists of a tandem repeat of a 10-bp motif, 5'-PuPuPuC(A/T)(T/A)GPYPyPy-3', which has been termed the p53-responsive element (23). Because PAb-246 interacts with the DNA binding domain of p53, Ab2 antibodies to PAb-246 might have structural similarity to p53 and, therefore, bind to the p53-responsive element. To detect such antibodies, we used a band shift assay using the labeled oligonucleotide of the p53-responsive element. Fig. 2A shows that Ab2 sera induced by PAb-246 were, indeed, able to cause a band shift. In contrast, Ab2 sera induced by immunization to the other Ab1 anti-p53 antibodies, or to R73, failed to do so. Thus, it seems that the Ab2 induced by PAb-246 were able to bind a p53-responsive DNA sequence like p53 itself (Fig. 2D).

To learn how specific this binding activity might be, we compared the band shift of the native sequence with that of an oligonucleotide modified by six base exchanges so that it is not recognized by the p53 protein (Fig. 2D). Fig. 2B shows that the Ab2 antibodies induced by immunization with PAb-246 could distinguish between the two oligonucleotides. Compared with the natural oligonucleotide sequence, the band shift of the modified oligonucleotide was reduced. However, p53 still was more specific and showed less binding of the modified oligonucleotide compared with the Ab2 sera (Fig. 2D). Thus, it seems that Ab2 antibodies are somewhat specific for the DNA sequence of the p53-responsive element, but not as specific as is p53 itself. To confirm that the DNA binding activity was indeed dependent on Ab2 anti-idiotypic antibodies, we tested whether the oligonucleotide binding could be inhibited by the addition of Ab1 PAb-246. As can be seen in Fig. 2C, addition of PAb-246 was found to completely abolish recognition of the p53-responsive element sequence. Therefore, it is likely that the Ab2 antibodies were anti-idiotypic to PAb-246, but could also specifically recognize the DNA sequence of the p53-responsive element.

Ab1 Antibodies Induce Ab3 Anti-p53 Antibodies. Mice receiving immunizations of the various Ab1 antibodies were tested for the spontaneous development of Ab3 anti-p53. As can be seen in Fig. 3A, the BALB/c or C57BL/6 mice that had been given immunizations of Ab1 PAb-240 or Ab1 PAb-246 produced antibodies to p53. Fewer of the mice given immunizations of Ab1 PAb-248 developed anti-p53 reactivity. Mice of either strain receiving immunizations of R73 did not develop a significant anti-p53 response (data not shown).

To confirm that the anti-p53-antibodies were actively induced Ab3 and not remnants of the injected Ab1 antibodies, we analyzed the isotypes of the anti-p53-antibodies. Although the monoclonal Ab1 antibodies used for induction were of the IgG1 isotype, the anti-p53 antibodies detected in the sera of the immunized mice were a mixture of different isotypes, dominated by IgG2b (Fig. 3B). Thus, the anti-p53 antibodies had to have been actively generated in response to the

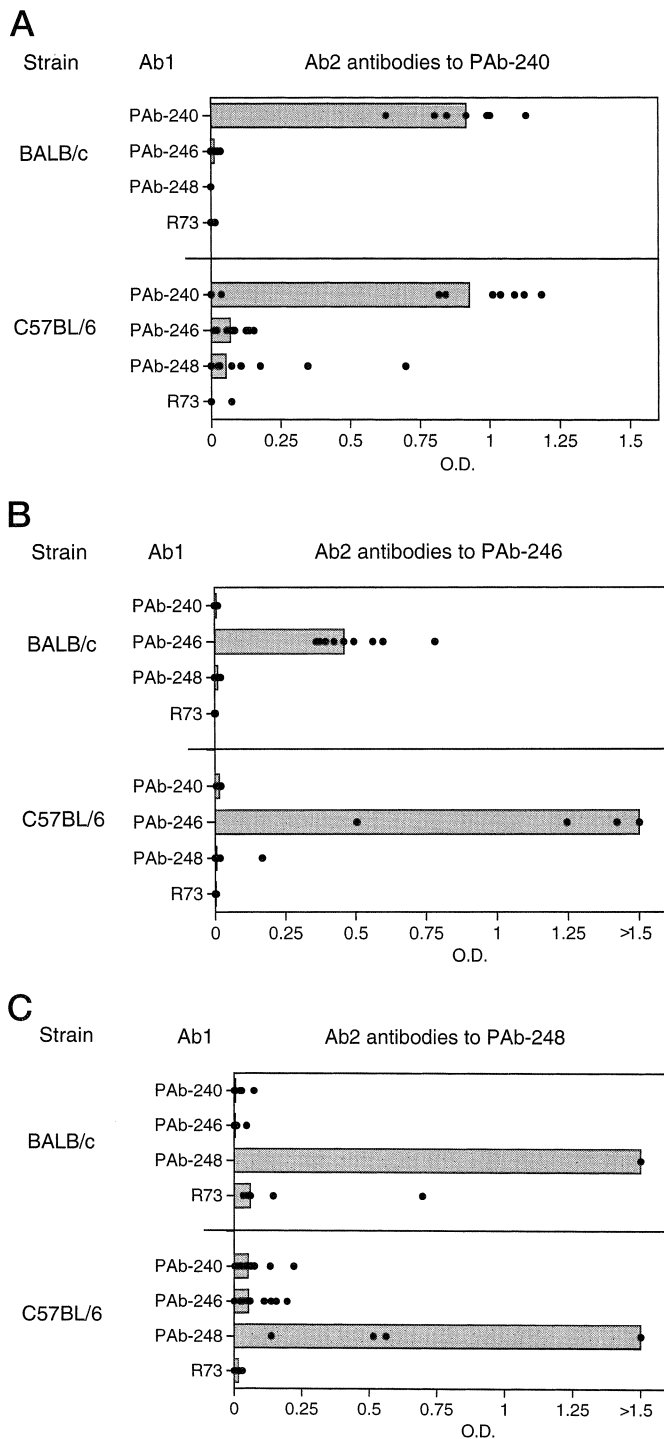


Fig. 1. Induction of anti-idiotypic antibodies (Ab2) by immunization with Ab1 anti-p53 antibodies. Groups of 10 mice were given immunizations to various monoclonal antibodies, and their Ab2 antibodies were measured by ELISA to the (Fab)₂ fragments of PAb-240 (A), of PAb-246 (B), or of PAb-248 (C). Compared with immunization with the control antibody R73, the production of Ab2 in mice given immunizations of the respective Ab1 antibodies was found to be statistically significant ($P < 0.002$).

Ab1 immunization. Moreover, the presence of different T cell-dependent IgG isotypes indicates that helper T cells probably play a role in the idiotypic network, since helper T cells are required for the IgG class switching of B cells (26).

Specificity of Ab3 Antibodies. To learn whether the Ab3 antibodies might preserve the p53-domain specificity of the inducing Ab1 antibodies, we studied the immunoprecipitation of p53 from cell

extracts of the tumor cell line Meth A (27), which overexpresses only mutant p53. We used an immunoprecipitation assay in this study because the ELISA assay cannot distinguish clearly between wild-type and mutant conformations of p53; adherence of p53 to the solid phase substrate apparently allows the molecule to assume the mutant as well as the wild-type conformation (data not shown).

PAb-240, which recognizes p53 in a mutant conformation, was able to precipitate mutant p53 from the Meth A cell extract (Fig. 4). Another anti-p53 monoclonal antibody, PAb-421, which recognizes the COOH-terminus of p53 (28), was also able to precipitate mutant p53. In contrast, PAb-246, which can only bind to p53 in its wild-type conformation, failed to precipitate mutant p53 from the Meth A cell extract. Fig. 4 also shows that mice receiving injections of Ab1 PAb-240, but not with Ab1 PAb-246 or Ab1 R73, produced Ab3 antibodies that could precipitate the mutant p53 protein. Thus, it seems that the Ab3 antibody induced by immunization to PAb-240 was, like PAb-240 itself, specific for mutant p53. In contrast to Ab1 PAb-240, immunization with Ab1 PAb-246 generated Ab3 that, like PAb-246, were specific for wild-type p53: the mice immunized with Ab1 PAb-246 were positive for anti-p53 in the ELISA assay (see Fig. 3), but failed to precipitate mutant p53 from the Meth A extract (Fig. 4).

Induction of the p53 Idiotypic Network by Peptides of CDR Domains of Ab1. We also tested whether BALB/c mice could be induced to produce Ab3 anti-p53 antibodies by immunization with synthetic peptides comprising the CDR2 or the CDR3 domains of the heavy or the light chains of PAb-240, PAb-246, or PAb-248 (see Table 1). Fig. 5 shows that Ab3 anti-p53 antibodies could, indeed, be obtained by immunizing mice with CDR peptides of PAb-240 or PAb-246, but not with CDR peptides of PAb-248.

Fig. 5 also shows that a CDR peptide of an Ab1 could be a more effective immunogen for an anti-p53 response than was the peptide epitope of the p53 antigen (Table 1) recognized by the Ab1 antibody:

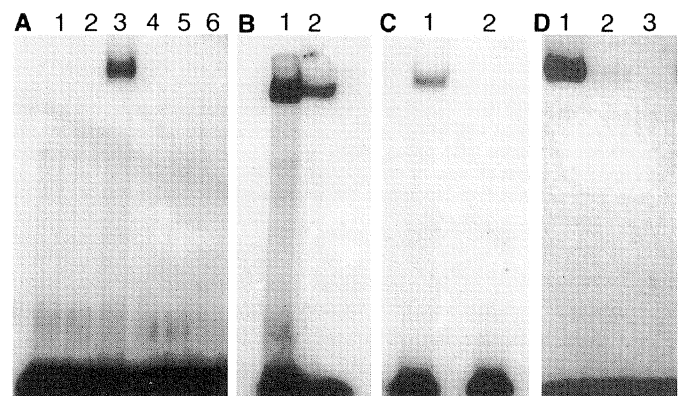


Fig. 2. Anti-idiotypic Ab2 antibodies induced by immunization with Ab1 PAb-246 bind to the oligonucleotide sequence of the p53-responsive element. A, Ab2 antibodies capable of binding to the DNA sequence of the p53-responsive element were detected by the induction of a band shift of the ³²P-labeled oligonucleotide by test sera: normal mouse serum (Lane 1) or sera induced by either immunization with PAb-240 (Lane 2), PAb-246 (Lane 3), PAb-248 (Lane 4), or the isotype-matched control antibody R73 (Lane 5). B, comparison of the band shift of different oligonucleotide sequences produced by Ab2 antibodies that had been induced by immunization with PAb-246. Lane 1 shows the band shift of an oligonucleotide of the wild-type sequence (TCGAGAGGCATGTCTAG-GCATGTCTC) and Lane 2 shows the band shift of a modified oligonucleotide sequence. C, Ab1 PAb-246 interferes with the recognition of the oligonucleotide sequence by Ab2 sera induced by immunization with PAb-246. The band shift of the p53-responsive oligonucleotide sequence by Ab2 serum is shown in Lane 1. Lane 2 shows the band shift produced by the same serum when 2 μ l of PAb-246 ascitic fluid was added to the reaction. D, the band shift induced by the p53 protein itself. Lane 1 shows the band shift of the wild-type oligonucleotide sequence produced by p53, which can be inhibited by the addition of PAb-246 (Lane 2). Lane 3 shows that p53 does not cause a band shift of the modified oligonucleotide sequence, which was used in B, Lane 2 (TTGAGGCAAG-GCAAGGCAAGGCAC).

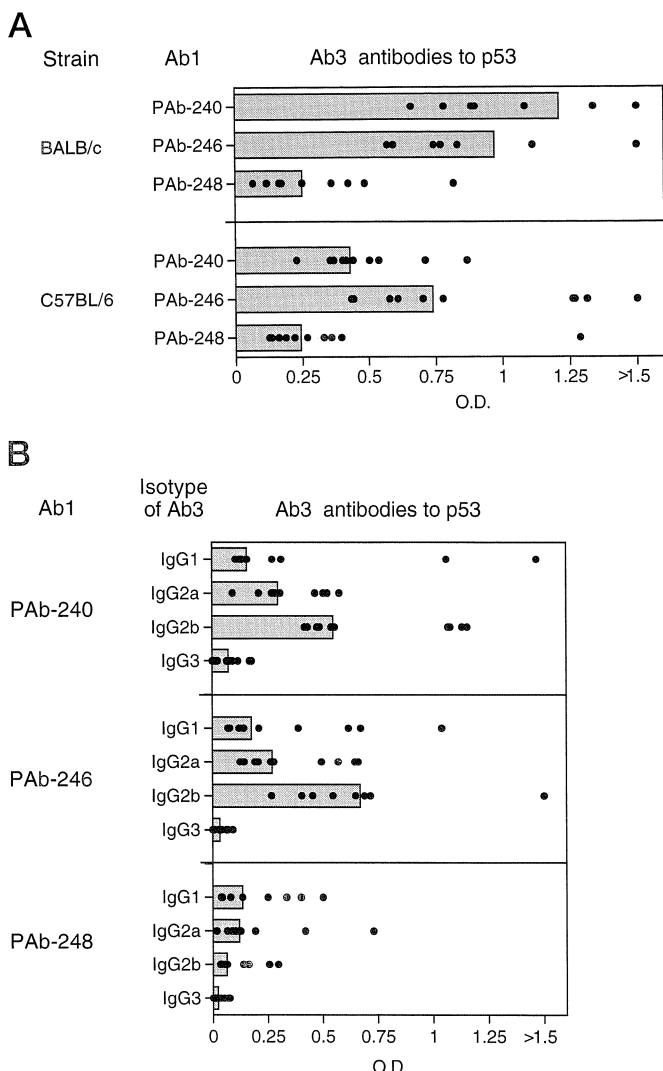


Fig. 3. The induction of Ab3 by immunization with Ab1 anti-p53 monoclonal antibodies. Mice received immunizations of Ab1 PAb-240, PAb-246, or PAb-248. The sera were then tested for the development of Ab3 anti-p53 antibodies by ELISA. Ab3 anti-p53 antibodies were detected with an IgG-specific secondary antibody (A) or with secondary antibodies specific for the IgG1, IgG2a, IgG2b, or IgG3 isotypes (B). The Ab1 antibodies used for immunizations all were of the IgG1-isotype. The specific induction of Ab3 by immunization with Ab1 PAb-240 or PAb-246 was statistically significant ($P < 0.002$).

the 20-mer peptide of p53 recognized by PAb-246 was much less immunogenic for the anti-p53 response than was the 20-mer VH CDR3 peptide of the PAb-246 antibody. Thus, idiotypic network immunization with an Ab1 may be more effective than is immunization with an epitope of the target antigen itself.

Ab1 Immunization Induces Resistance to Tumor Metastasis. Since immunization with a suitable Ab1 antibody is capable of inducing an immune response to p53, we tested whether this response might be associated with resistance to tumor challenge. Using the spontaneous metastasizing 3LL lung carcinoma (24), which overexpresses p53, C57BL/6 mice, syngeneic with the 3LL tumor, were given immunizations of PAb-240, PAb-246, PAb-248, or the control antibody R73, and inoculated with 3LL cells. The tumors were removed when they reached a size of 8 mm, and 21 days later the mice were sacrificed, and their lungs were weighed to measure the mass of lung metastases. Fig. 6 shows the result of a representative experiment. Immunization with PAb-240 was able to significantly reduce the metastases, whereas immunization with the R73 control antibody or with PAb-248, which does not efficiently induce Ab3 anti-p53

antibodies, failed to do so. Immunization with PAb-246 was less effective than was PAb-240, but still conferred significant protection from lung metastasis. Thus, it can be concluded that the activation of a p53-based idiotypic network by immunization with Ab1 may be associated with resistance to metastasis by a tumor cell line.

DISCUSSION

Immunization with a particular Ab1 has often been observed to generate Ab2 anti-idiotypic antibodies, but rarely are Ab3 anti-anti-idiotypic antibodies produced in the same animal. In this study, we found that all three Ab1 anti-p53 antibodies could induce specific Ab2 anti-idiotypic antibodies. However, only two of the Ab1 antibodies, PAb-240 and PAb-246, were efficient inducers of Ab3 anti-p53 antibodies. Thus, the anti-p53 network seems to have a bias for certain Ab1 antibodies.

The Ab2 antibodies induced by Ab1 PAb-246 demonstrated unprecedented structural specificity for DNA; they showed some similarity to the structure of the p53 DNA binding domain, in that they could specifically bind the DNA oligonucleotide sequence of the p53-responsive element, and could discriminate between the wild-type oligonucleotide and an oligonucleotide “mutated” at six positions. The specificity of the antibodies, however, seemed to be more degenerate than the specificity manifested by p53 itself, which does not retard at all the mutated oligonucleotide in the gel shift assay (Fig. 2).

The Ab3 antibodies preserved the specificity of the Ab1 inducers: Ab3 induced by PAb-246 recognized the wild-type p53 molecule, whereas Ab3 induced by PAb-240 recognized mutant p53.

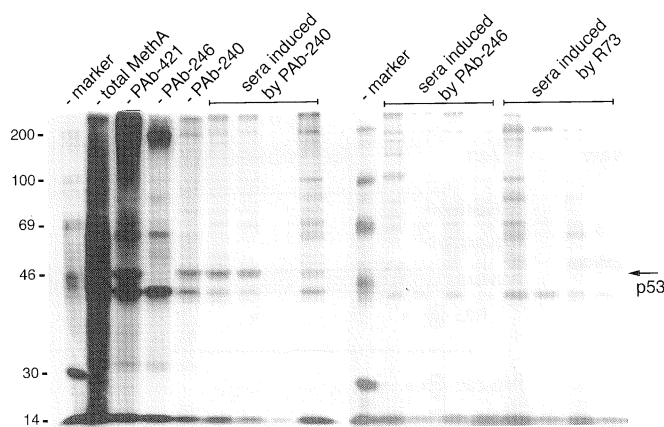


Fig. 4. Immunoprecipitation of mutant p53 from Meth A cell extracts. Mice received immunizations of Ab1 PAb-240, PAb-246, or R73. Their sera were then tested for the presence of Ab3 antibodies specific for mutant p53 by immunoprecipitation from Meth A cells, which only express p53 in a mutant conformation. Four representative individual sera induced by immunization with each Ab1 antibody were compared with monoclonal anti-p53 antibodies, PAb-240, PAb-246 and PAb-421, in their ability to precipitate the mutant p53 from Meth A cells. Only test sera from PAb-240-immunized mice and the monoclonal antibodies PAb-240 and PAb-421 could precipitate mutant p53 (arrow).

Table 1 Sequences of the CDR peptides of Ab1 anti-p53 antibodies and of the p53 peptide epitope recognized by PAb-246 (16)

Ab1 antibody	Chain/CDR region	Sequence
PAb-240	VH CDR2	GEIDPDSYTNYNQNFKDKA
	VH CDR3	YYCARLLRYFAMDYWGQGT
	VL CDR3	YYCQHIRELTRSEGGPS
PAb-246	VH CDR2	GDINPNNGYTTYNQKVKGKA
	VH CDR3	CVRGGGLKGYFPVYWGQGT
	VL CDR3	ATYYCQQRSSFPPFTYGSSTK
PAb-248	VH CDR2	GDYYPNNGFTTYNQKFKGKA
	VH CDR3	AVYYCARSGSRFDYWGQGT
	VL CDR3	VYFCQQSNWVHARGGGTK
PAb-246 epitope		PLSSFVPSQKTYQGNYGFHLG

Some CDR peptides of Ab1 PAb-240 or PAb-246 sufficed to generate Ab3 anti-p53 antibodies. Because these CDR peptides are linear sequences of at most 20 amino acids, it was very unlikely that the peptides could have presented with significant stability the folded conformations of the antigen-binding clefts of the intact Ab1 antibody molecules (11). The Ab1 CDR peptides, thus, were not likely to have initiated *de novo* Ab3 antibody responses specific for particular conformations of p53. Therefore, a latent, preprogrammed idiotypic network centered around PAb-240 and PAb-246 may have been activated by the peptide immunization (29). It has been observed that natural idiotypic networks may be centered around only certain self-molecules, and may be focused on the part of the self-molecule that has significant biological function (29). Thus, p53 may belong to the set of dominant self antigens constituting the immunological homunculus (29).

Note that the p53 idiotypic network not only involves the wild-type p53 conformation, but also the mutant conformation of p53 recognized by PAb-240. Different point mutations of the *p53* gene may induce the same, standard conformational change of the p53 protein, which has been associated with recognition by the PAb-240 antibody and by loss of recognition by PAb-246 (14). It has been suggested that the p53 conformation detected by PAb-240 might not necessarily be restricted to mutant p53, but rather might be a natural conformation variant of p53 with distinct physiological functions (30).

What could be the physiological relevance of a natural p53 idiotypic network? Mice bearing tumors make anti-p53 antibodies (31), and human cancer patients have been shown to produce antibodies (32) and T cells (33) to p53. It is conceivable that immune recognition of p53, both mutant and wild-type, could play a role in tumor immunosurveillance. Indeed, our finding that immunization to Ab1 PAb-240, and to a lesser degree Ab1 PAb-246, induced resistance to metastasis of the 3LL tumor line is compatible with a role of the p53 idiotypic network in tumor surveillance.

The antitumor effect of some antibodies (34) has been explained by direct cytotoxicity or antibody-dependent cell-mediated cytotoxicity (35, 36). However, it has also been reported that antibodies can

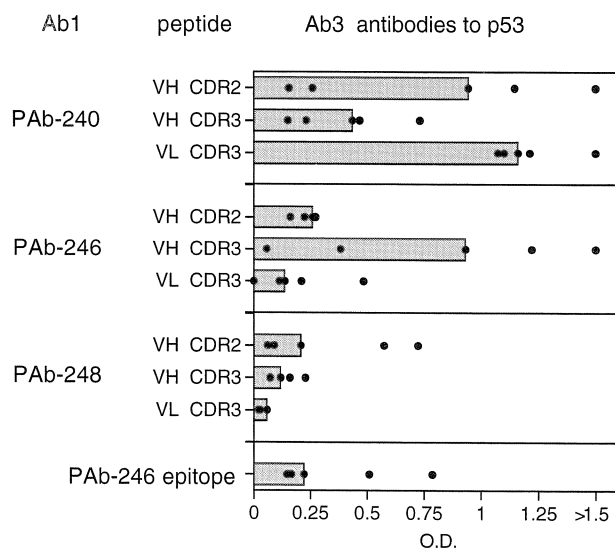


Fig. 5. CDR peptides of Ab1 antibodies induce Ab3 anti-p53. Mice were immunized with CDR peptides from the light (VL) and heavy (VH) chains of Ab1 antibodies. The sera were then tested for the development of Ab3 anti-p53 antibodies. A peptide derived from p53, which is the epitope of PAb-246 (indicated as "PAb-246-epitope") was also used. The induction of Ab3 antibodies by the VH CDR3 peptide of Ab1 PAb246 ($P < 0.02$) and by the VL CDR3 peptide of Ab1 PAb-240 ($P < 0.01$) were found to differ significantly from the response to the respective CDR peptides from Ab1 PAb-248. The responses to the other CDR peptides and the p53-derived peptide (PAb-246 epitope) did not differ significantly from the negative response to the PAb-248-derived CDR peptides.

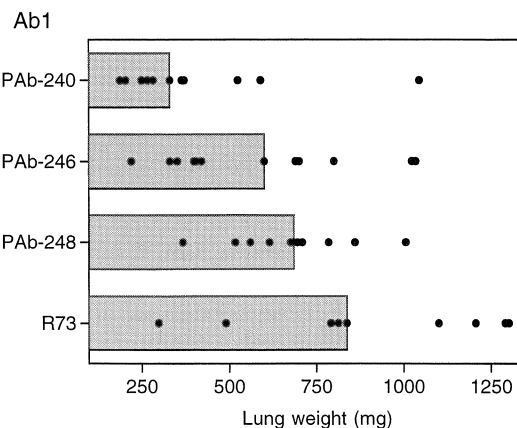


Fig. 6. Effect of Ab1 immunization on 3LL tumor metastasis. C57BL/6 mice were immunized with Ab1 PAb-240, PAb-246, PAb-248, or R73. After boosting, the mice were challenged with the syngeneic 3LL lung carcinoma (2×10^5 cells in one hind foot pad). The lung weight was determined as a measure of metastases 3 weeks after excision of the primary tumors. Immunization with Ab1 PAb-240 ($P < 0.005$) or PAb-246 ($P < 0.05$) significantly reduced lung metastasis compared with immunization with Ab1 R73. In contrast, immunization with PAb-248 did not result in a significant reduction of lung metastases. Similar results were obtained in the three experiments done.

synergize with CTLs in tumor cell killing (37). Since p53 is an intracellular antigen, it is likely that the protective effect against 3LL metastases was not entirely due to the induced antibodies, but perhaps also to p53-specific CTLs. Indeed, we find that immunization with Ab1 CDR peptides may significantly enhance the ability of BALB/c splenocytes to lyse Meth A tumor cells (15). It is conceivable that p53-specific CTL precursors were recruited in the course of the idiotypic response. This view is supported by the finding of Röpke *et al.* (38), who could isolate wild-type p53-specific CTL from the blood of a healthy, tumor-free donor that were able to kill tumor cell lines overexpressing p53. Our finding of Ab3 antibody induction by Ab1 CDR-peptide immunization suggests the participation of T cells in the p53 idiotypic network, because T cells recognize processed linear peptides presented by MHC molecules. Moreover, the IgG isotypes of the Ab3 anti-p53 antibodies were T cell-dependent. Further work will be needed to isolate and characterize helper and cytotoxic T cells and to define the mechanism of the antimetastatic activity.

It has been reported that the prognosis of cancer patients is poor when the patients spontaneously manifest antibodies to the N-terminal domain of p53 (39). As shown here, Ab1 PAb-248, which is specific for the N-terminal domain, was also not effective in our mouse model. It would seem that immunity to other domains, such as those recognized by PAb-240 and PAb-246, may be more effective. It remains to be seen whether individuals responding to these domains might indeed enjoy a better prognosis.

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