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Immunology

Epstein–Barr virus/complement fragment C3d receptor (CR2) reacts with p53, a cellular antioncogene-encoded membrane phosphoprotein: Detection by polyclonal anti-idiotypic anti-CR2 antibodies

(complement receptor type 2/post-membrane signals)

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ABSTRACT **Epstein–Barr virus and the C3d fragment of the third component of complement are specific extracellular ligands for complement receptor type 2 (CR2). However, intracellular proteins that react specifically with CR2 and are involved in post-membrane signals remain unknown. We recently prepared polyclonal anti-idiotypic anti-CR2 antibodies (Ab2) by using the highly purified CR2 molecule as original immunogen. We showed that Ab2 contained anti-idiotypic specificities that mimicked extracellular domains of CR2 and detected two distinct binding sites on CR2 for its specific extracellular ligands, Epstein–Barr virus and C3d. We postulated that Ab2 might also contain specificities that could mimic intracellular domains of CR2. Here we report that Ab2, which did not react with Raji B-lymphoma cell surface components, detected specifically, among all components solubilized from Raji cell membranes, a single intracellular membrane protein of apparent molecular mass of 53 kDa. This protein was identified as the p53 cellular antioncogene-encoded membrane phosphoprotein by analyzing its antigenic properties with Pab1801, a monoclonal anti-p53 antibody, and by comparing its biochemical properties with those of p53. Additionally, solubilized and purified CR2 bound to solubilized p53 immobilized on Pab1801-Sepharose. p53, like CR2, was localized only in purified plasma membranes and nuclei of Raji cells. These data suggest strongly that p53, a cellular antioncogene-encoded phosphoprotein, reacted specifically with CR2 in Raji membranes. This interaction may represent one of the important steps through which CR2 could be involved in human B-lymphocyte proliferation and transformation.**

Complement receptor type 2 (CR2), which serves as the receptor for Epstein–Barr virus (EBV), as well as for the C3d fragment of the third component of complement, was identified as a 140-kDa membrane glycoprotein, gp140, purified from the cell surface of the human B-lymphoma line Raji (1, 2). CR2 is involved in human B-lymphocyte activation (3). Indeed, crosslinking of CR2 at the cell surface by specific ligands such as polyclonal anti-CR2 $F(ab')_2$ fragments (4), anti-CR2 monoclonal antibody (mAb) OKB-7 (5), particle-bound C3d (6), or UV-inactivated EBV particles (7) led to the enhancement of B-cell proliferation in synergy with T-cell-derived factors, such as B-cell growth factor (3). Activation of cultured human peripheral B lymphocytes by extracellular ligands such as anti-IgM or *Staphylococcus aureus* Cowan I (8) or human tonsillar B lymphocytes by phorbol 12-myristate 13-acetate, an activator of intracellular protein kinase C (9), induced phosphorylation of cellular CR2. Plasma membrane CR2 was phosphorylated on serine and tyrosine residues when immobilized on anti-CR2 mAb (10) or when present in purified subcellular fractions of Raji cells (11). Phosphorylated CR2 was also localized in purified nuclei of Raji cells (10). Solubilized nuclear CR2 reacted with a nuclear phosphoribonucleoprotein, p120 RNP, that was phosphorylated on serine residues (10, 11). Cell-free phosphorylation of p120 RNP was dependent of the presence of CR2 (10). Moreover, phosphorylation of plasma membrane CR2 or of nuclear CR2 and p120 RNP was significantly increased when human iC3b/C3d or OKB-7 reacted specifically with CR2 in purified subcellular fractions (11). Crosslinking of CR2 on human B lymphocytes induced increase of Ca^{2+} flux, but only in synergy with crosslinked membrane IgM (12), as crosslinking of multivalent ligands on human B cells activated protein kinase C and phosphatidylinositol phosphate pathways (13–15). However, the mechanisms by which post-membrane signals were transmitted after crosslinking of CR2 at the human B-lymphocyte surface, and the identity of the intracellular proteins reacting with CR2, remained unknown.

To analyze interactions between CR2 and intracellular components, we postulated that polyclonal anti-idiotypic anti-CR2 antibodies (Ab2), raised against $F(ab')_2$ fragments of polyclonal anti-CR2 antibodies (Ab1) prepared against solubilized and highly purified CR2 (gp140), could carry specificities that mimic transmembrane and/or intracellular domains of CR2 reacting with intracellular compounds. Indeed, we have recently shown that Ab2 could be used to analyze interactions between CR2 and specific extracellular ligands such as human C3d and EBV (16). Ab2 detected distinct binding sites for human C3d and EBV on the extracellular domains of CR2. Indeed, the C3d-binding specificities contained in Ab2 (C3d/Ab2) reacted only with C3d but not with EBV (16). The presence of multiple or distinct binding sites on CR2 for its two ligands was also recently supported by Tanner *et al.* (17), who induced Val-Glu deletions in the 6-amino acid domain homologous to the putative C3d-binding domain (18) of gp350/220, the virus capsid glycoprotein that mediates EBV binding to CR2.

We report here that some Ab2 specificities reacted specifically, among all solubilized proteins from Raji cells, with a single intracellular membrane protein, characterized by an apparent molecular mass of 53 kDa, p53. This p53 intracellular membrane protein was identified by its antigenic and

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Abbreviations: EBV, Epstein–Barr virus; C3d, cleavage product of third complement component; CR2, complement receptor type 2 (EBV/C3d receptor); mAb, monoclonal antibody; Ab1, polyclonal anti-CR2 antibodies; Ab2, polyclonal anti-idiotypic anti-CR2 antibodies; NP-40, Nonidet P-40; IEF, isoelectric focusing.

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biochemical properties as the p53 cellular antioncogene-encoded phosphoprotein described by others. Furthermore, interaction of purified CR2 with solubilized p53 was demonstrated.

MATERIALS AND METHODS

Human Cells. Human cell lines used were Raji, a CR2-positive B-lymphoma cell line; MOLT-4 and CEM, CR2-positive and CR2-negative T-lymphoma cell lines, respectively; U-937, a monocytic cell line; and SK-MEL-170, a melanoma cell line. All human cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 37°C in a 5% CO_2 incubator. Leukocytes and erythrocytes were isolated from citrated blood obtained from normal donors as described (19).

Preparation of Ab1 and Ab2. To obtain Ab1, a rabbit was immunized with gp140, the highly purified CR2 obtained by affinity chromatography on C3b/iC3b-Sepharose (20). Ab2 were prepared using $F(ab')_2$ fragments of Ab1 IgG fractions as immunogen. C3d/Ab2 specificities present in Ab2 were isolated by affinity chromatography on iC3b/C3d-Sepharose (16).

mAbs. Pab1801, a hybridoma cell supernatant recognizing human p53, was kindly provided by L. V. Crawford (Imperial Cancer Research Fund, Cambridge, U.K.). An anti-C3d mAb prepared in our laboratory (unpublished data) was used as irrelevant antibody.

Subcellular Fractionation of Raji Cells. Raji subcellular fractions were prepared by differential centrifugation following the method of McKeel and Jarett (21) as modified by Delcayre *et al.* (10).

Preparation of Membrane Extracts. Human cells (2×10^9) were lysed by addition of 0.3 mM barbital buffer (pH 7.4; conductance, 4 mS) at 4°C. After removal of nuclei by centrifugation at $300 \times g$ for 15 min at 4°C, total membranes were collected by centrifugation at $100,000 \times g$ for 30 min at 4°C. Membrane components were solubilized for 45 min at 4°C in 10 ml of 0.3 mM barbital buffer (pH 7.4) containing 1% (vol/vol) Nonidet P-40 (NP-40), 2% (vol/vol) glycerol, and 0.5 mM phenylmethylsulfonyl fluoride. Insoluble material was removed by centrifugation at $100,000 \times g$ for 30 min at 4°C. The protein content of the solubilized components was determined by the Bradford assay (22) and adjusted to 4 mg/ml.

Radiolabeling. Purified CR2 (500 ng), solubilized proteins (400 μg), or Raji cells (10^8 cells per ml) were labeled with ^{125}I (185 MBq, carrier-free; Amersham) by using immobilized Iodo-Gen (Pierce) as described by the manufacturer. The specific activity achieved was 2×10^5 cpm/ng for CR2 and 10^6 cpm/μg for solubilized proteins.

Immunoblotting. Solubilized membrane proteins (200 μg) were submitted to SDS/PAGE (0.1% SDS, 7.5% polyacrylamide gel containing 0.8% bisacrylamide; ref. 23) and then transferred electrophoretically to nitrocellulose sheets (Schleicher & Schuell) by use of Transblot apparatus (Bio-Rad). Blotting with IgG fraction of the various antisera was performed in the presence of 150 mM NaCl, as described (20).

Immunoprecipitation. Solubilized proteins from unlabeled (1 mg) or labeled (10^6 cpm) membranes were incubated for 1 hr at 4°C with 3 mg of IgG of rabbit nonimmune serum covalently bound to 250 mg of Sepharose 4B (16). Effluents were incubated for 2 hr at 4°C with 1 mg of IgG of the antisera (Ab1 or Ab2) or hybridoma supernatants (Pab1801 or anti-C3d) covalently bound to 100 mg of Sepharose 4B (16). After the immunobeads were extensively washed, proteins were eluted in SDS buffer by heating for 2 min at 100°C and analyzed by SDS/PAGE and autoradiography using Trimax-XD films (3M Co.) in the presence of intensifying screens. Reference molecular mass markers used were factor H (150 kDa; purified in our laboratory; ref. 20), phosphorylase *b* (92 kDa; Sigma), bovine serum albumin (68 kDa; Sigma), and carboxypeptidase B (35 kDa; Sigma).

Isoelectric Focusing (IEF). Solubilized membrane proteins (1 mg) from unlabeled Raji cells were mixed with IEF sample buffer containing 8 M urea, 1% NP-40, and 4% (wt/vol) Ampholines (pH 3.5–10; LKB). IEF was performed in vertical polyacrylamide slab gels as described by De Cordoba *et al.* (24). The electrode solutions were 0.02 M NaOH for the upper (cathode) reservoir and 0.01 M H_3PO_4 for the lower (anode) reservoir. Gels were run at a constant current of 10 mA, with the maximum voltage of 400 V reached in 15 hr at room temperature. For two-dimensional gels, lanes from the IEF gels were cut out and placed on top of SDS/7.5% polyacrylamide slab gels (23).

***In Vitro* Phosphorylation Assay.** Solubilized antigens immobilized on specific antibodies bound to Sepharose were incubated in 50 μl of 20 mM Tris·HCl, pH 7.5/100 mM NaCl/25 mM $MnCl_2$ or $MgCl_2$/0.2% NP-40. Phosphorylation was started by addition of 10 μM [γ-^{32}P]ATP (specific activity, 185 GBq/mmol; Amersham) and allowed to proceed for 20 min. Then the immunobeads were extensively washed and phosphorylated antigens were analyzed by SDS/PAGE and autoradiography.

RESULTS

Specific Binding of Ab2 to p53, an Intracellular Membrane Component. We used Ab2 (16) to detect intracellular CR2-binding proteins. Indeed, as Ab2 carried specificities that mimicked extracellular binding sites of CR2 for its two specific external ligands, EBV and human C3d, we postulated that some Ab2 specificities mimicked also internal CR2 binding domains for intracellular ligands. Thus, Ab2 specificities were tested in immunoblotting experiments with total membrane extracts prepared from Raji cells in 1% NP-40. Ab2 IgG detected, among all the solubilized membrane proteins immobilized on nitrocellulose, a single polypeptide chain of apparent molecular mass 53 kDa, p53 (Fig. 1*A*, lane 3). This apparent molecular mass was not modified in reducing conditions (data not shown). IgG fractions of the nonimmune serum (lane 1) or C3d/Ab2, the C3d-binding specific-

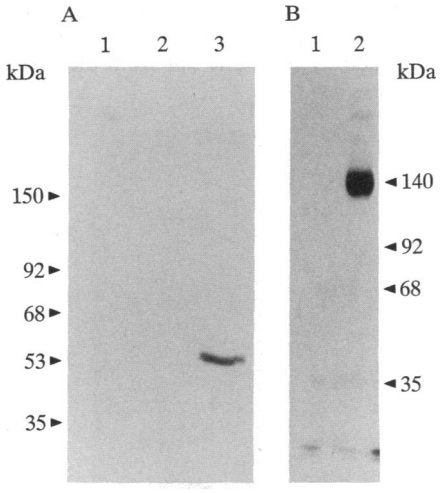

FIG. 1. Detection of membrane proteins by Ab2 IgG. Solubilized membrane proteins from Raji cells, either unlabeled (*A*) or ^{125}I-labeled after external cell-labeling with Iodo-Gen (*B*), were subjected to SDS/PAGE. (*A*) Immunodetection of unlabeled proteins transferred onto nitrocellulose was performed with IgG from nonimmune serum (lane 1), from C3d/Ab2 (lane 2), or from Ab2 (lane 3). (*B*) Immunoprecipitation of labeled proteins was performed with IgG fractions of Ab2 (lane 1) or Ab1 (lane 2).

ities contained in Ab2, mimicking the external domain of CR2 and reacting only with human C3d (16), did not react with p53 (lane 2) or with any other membrane components. We had already shown that Ab2 did not bind to the Raji cell surface (16). Additionally, Ab2 did not immunoprecipitate any ^{125}I-labeled cell surface membrane protein (Fig 1*B*, lane 1), whereas Ab1, used as control, immunoprecipitated CR2 (lane 2).

Thus, some Ab2 specificities reacted specifically with p53, an intracellular membrane protein.

Subcellular Distribution of p53. Plasma membrane, cytosolic, and nuclear fractions of Raji cells were purified as described (10), and proteins of these fractions were solubilized or incubated in 1% NP-40 and analyzed by immunoblotting using Ab2 IgG. p53 was localized, as shown for CR2 (10), in plasma membrane and nuclear fractions of Raji cells, but not in cytosol (Fig. 2).

Specificity of CR2 Binding to p53. To analyze the specificity of CR2 binding to p53, we studied the interaction of highly purified, ^{125}I-labeled CR2 with solubilized p53 immunoprecipitated on Ab2. Indeed, Ab2 recognized not only p53 immobilized on nitrocellulose but also the solubilized form of p53—i.e., when total membrane components solubilized from Raji cells were labeled with ^{125}I, Ab2 immunoprecipitated ^{125}I-labeled p53 in either the presence (Fig. 3*A*, lane 1), or absence (lane 2) of 150 mM NaCl, whereas C3d/Ab2 did not recognize this antigen (lane 3). Thus, interactions between purified CR2 and p53 were analyzed: ^{125}I-labeled purified CR2 reacted specifically, in the absence of NaCl, with unlabeled p53 immobilized on Ab2-Sepharose (Fig. 3*B*, lane 3). The interaction between p53 and ^{125}I-CR2 was modified by NaCl concentration: the amount of ^{125}I-CR2 bound to p53 was lower in the presence of 50 mM NaCl (Fig. 3*B*, lane 4) than in the absence of NaCl and was completely abolished in the presence of 150 mM NaCl (lane 5). Whatever the NaCl concentration, ^{125}I-CR2 did not bind to Ab2-Sepharose in the absence of p53 (lane 1) or to C3d/Ab2-Sepharose incubated with p53 (lane 2). As the binding of p53 to Ab2 was not modified by 150 mM NaCl, these data suggested strongly that the interactions between CR2 and p53 represented low-affinity protein–protein interactions.

Identification of p53, the Intracellular CR2-Binding Protein. The plasma membrane and nuclear localization of p53 detected with Ab2 prompted us to identify this protein by using mAbs raised against different intracellular membrane proteins characterized by the same apparent molecular mass of 53 kDa and described as markers for cell transformation (25). Among these mAbs, Pab1801 reacted with p53 recognized by Ab2. Pab1801 was prepared by others against the human p53 cellular antioncogene-encoded protein (26). First, Pab1801 did not bind to the Raji cell surface, as already described for other cells (26), when compared to the binding of OKB-7, an anti-CR2 mAb, in our cell surface binding assay (19). Second, sequential adsorptions and elutions of membrane proteins solubilized from Raji cells and labeled with ^{125}I were per-

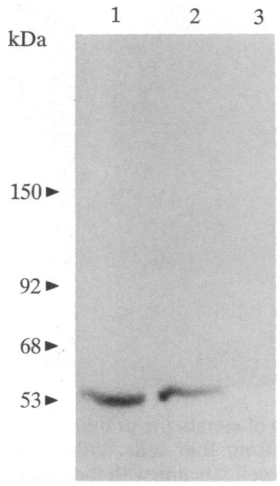

FIG. 2. Subcellular distribution of p53 in Raji cells. Solubilized proteins from purified membrane (lane 1), nuclear (lane 2), or cytosolic (lane 3) fractions were analyzed by immunoblotting with Ab2 IgG.

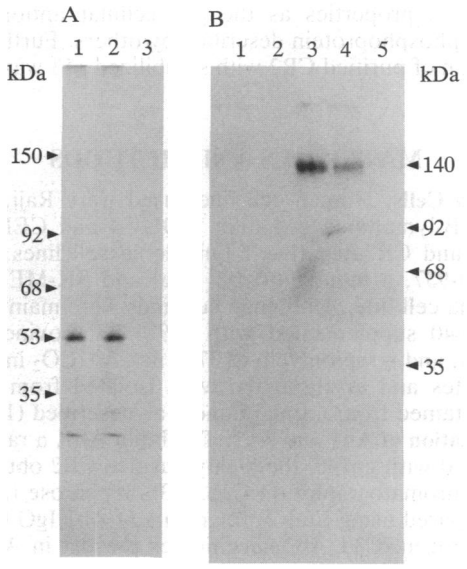

FIG. 3. Specificity of CR2 binding to p53. (*A*) Solubilized membrane proteins from Raji cells were ^{125}I-labeled and then immunoprecipitated with Ab2 IgG in the presence (lane 1) or absence (lane 2) of 150 mM NaCl or with C3d/Ab2 (lane 3). (*B*) Binding of purified ^{125}I-labeled CR2 was assayed with p53 bound on Ab2-Sepharose in the absence of NaCl (lane 3) or in the presence of 50 mM NaCl (lane 4) or 150 mM NaCl (lane 5). Nonspecific binding of purified ^{125}I-labeled CR2 was tested in the absence of NaCl, either with p53 incubated with C3d/Ab2-Sepharose (lane 2) or with Ab2-Sepharose incubated without p53 (lane 1). Proteins were eluted in sample buffer under reducing conditions and analyzed by SDS/PAGE and autoradiography.

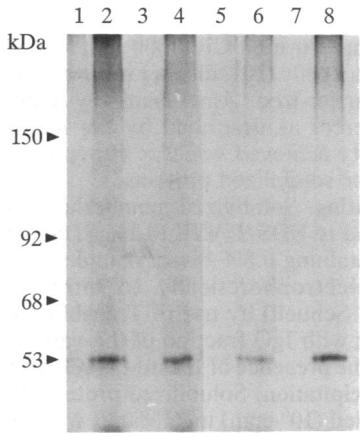

FIG. 4. Identification of p53. Solubilized membrane proteins from Raji cells were ^{125}I-labeled and then immunoprecipitated with IgG fractions of nonimmune serum (lane 1), Ab2 (lane 2), anti-C3d mAb (lane 3), or Pab1801 (lane 4). ^{125}I-labeled proteins eluted from Ab2 were bound to and eluted from anti-C3d mAb (lane 5) or Pab1801 (lane 6) covalently bound to Sepharose. ^{125}I-labeled proteins eluted from Pab1801 were bound to and eluted from IgG fractions from C3d/Ab2 (lane 7) or Ab2 (lane 8) covalently bound to Sepharose. Proteins were eluted in sample buffer under reducing conditions and analyzed by SDS/PAGE and autoradiography.

formed on IgG of either Ab2 or Pab1801 bound covalently to Sepharose. ^{125}I-labeled p53 eluted from Ab2-Sepharose (Fig. 4, lane 2) bound specifically to Pab1801 mAb (lane 6) but not to an irrelevant anti-C3d mAb (lane 5). Identically, ^{125}I-labeled p53 eluted from Pab1801-Sepharose (lane 4) bound specifically to Ab2 (lane 8) but not to C3d/Ab2 (lane 7). p53 did not react with nonimmune serum IgG (lane 1) or with irrelevant anti-C3d mAb (lane 3).

Cellular Distribution of p53 Recognized by Ab2. Expression of the p53 antioncogene-encoded protein recognized by Pab1801 has been described in various murine or human cell lines transformed chemically or virally (27). Therefore, cellular distribution of p53 recognized by Ab2 specificities was analyzed in membrane extracts prepared from various CR2-positive or -negative human cell lines. Ab2 detected the presence of p53 in the CR2-positive T-cell line MOLT-4, in the CR2-negative T-cell line CEM, in the CR2-negative monocytic cell line U-937, and in the CR2-negative melanoma cell line SK-MEL-170 (Fig. 5). In the monocytic cell line U-937, a doublet of 53/55 kDa was detected by Ab2. Identical data were obtained with Pab1801. p53 was not detected by Ab2 in normal human erythrocytes, polymorphonuclear cells, or lymphocytes (data not shown).

Biochemical Properties of p53. Additional biochemical properties of p53 recognized by Ab2 were compared with those of p53 identified by Pab1801. IEF followed by SDS/PAGE showed that p53 recognized by Ab2 was characterized by an isoelectric point of 7.2 (Fig. 6).

Furthermore, cell-free phosphorylation of p53 was performed as already described for CR2 (10). When the p53 recognized by Ab2 was immunoprecipitated on Pab1801 and incubated in a phosphorylation reaction mixture with [γ-^{32}P]ATP, p53 was phosphorylated in the presence of $MnCl_2$ but not in the presence of $MgCl_2$ (Fig. 7).

DISCUSSION

We undertook to identify intracellular membrane components that react with CR2 in the human B-lymphoma cell line Raji. For this purpose, we used Ab2, anti-idiotypic antibodies prepared by immunizing a rabbit with purified F(ab')$_2$ fragments of Ab1 antibodies generated against highly purified CR2 (gp140) solubilized from Raji plasma membranes. Ab2 carried specificities that mimicked extracellular domains of CR2 and detected two distinct binding sites, one for human C3d and the other for EBV, the two specific extracellular ligands of CR2 (16). Thus, we postulated that Ab2 might also carry specificities that mimicked CR2 intracellular domains.

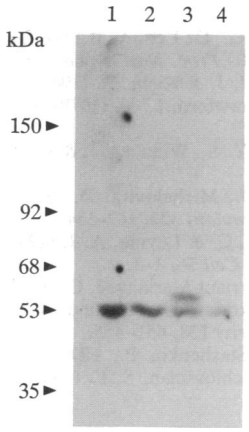

FIG. 5. Cellular distribution of p53 detected by Ab2 IgG. Solubilized membrane components extracted from MOLT-4 (lane 1), CEM (lane 2), U-937 (lane 3), or SK-MEL-170 (lane 4) cell lines were analyzed by immunoblotting with Ab2 IgG.

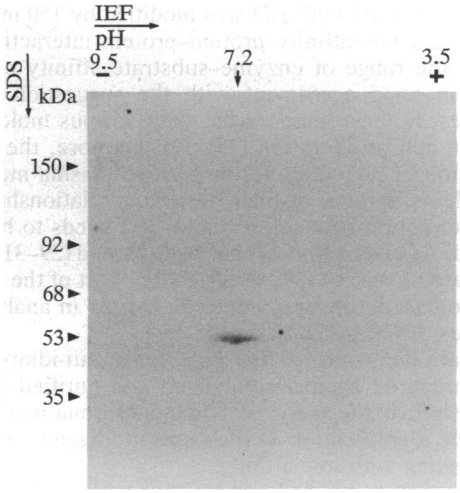

FIG. 6. Determination of p53 isoelectric point. Solubilized membrane proteins from Raji cells were analyzed by two-dimensional IEF–SDS/PAGE followed by immunoblotting with Ab2 IgG.

Analysis of Ab2 reactivity with Raji plasma membrane extracts detected a single membrane protein, whose apparent molecular mass was 53 kDa in the presence or absence of reducing agents. This p53 membrane antigen detected by Ab2 was not localized on the external side of Raji plasma membranes: (*i*) Ab2 did not bind to the Raji cell surface (16) and did not immunoprecipitate any external ^{125}I-labeled membrane proteins; (*ii*) the C3d-binding specifities contained in Ab2 (C3d/Ab2), which mimicked the CR2 extracellular domain reacting with the external C3d ligand, did not recognize any extracellular or intracellular membrane protein; (*iii*) Pab1801, a mAb specific for the p53 antioncogene product, did not react with the Raji cell surface.

The specificity of the interaction between p53 and CR2 was supported by the specific recognition of p53 by Ab2 and was demonstrated by the ability of ^{125}I-labeled CR2 to bind specifically to p53 solubilized and immobilized on Ab2-Sepharose or on Pab1801-Sepharose. Interaction of Ab2 with p53 was not modified by 150 mM NaCl, a concentration that does not affect antigen–antibody interactions. However,

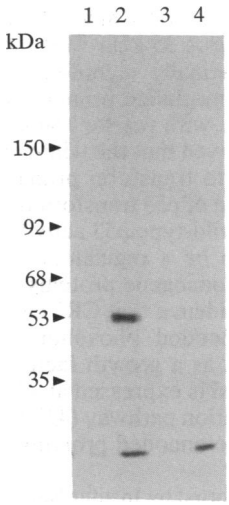

FIG. 7. *In vitro* phosphorylation of p53. Solubilized membrane proteins from Raji cells were immunoprecipitated either with IgG of anti-C3d mAb (lanes 1 and 3) or with Pab1801 (lanes 2 and 4) in the presence of $MnCl_2$ (lanes 1 and 2) or $MgCl_2$ (lanes 3 and 4). Then [γ-^{32}P]ATP was added for 20 min at 20°C. Proteins were eluted in sample buffer under reducing conditions and analyzed by SDS/PAGE and autoradiography.

interaction of CR2 with p53 was modified by 150 mM NaCl, supporting a low-affinity protein–protein interaction, most likely in the range of enzyme–substrate affinity constants. This is in good agreement with the suggestion that p53 associates, at least temporarily, with various molecules involved in cell proliferation (28). Furthermore, the identical localization of p53 and CR2 in purified plasma membranes and nuclei is in favor of their functional relationship in Raji cells. Interaction between p53 and CR2 needs to be further analyzed. As cDNA for CR2 has been cloned (29–31), the use of truncated forms of CR2 lacking all or part of the 35-amino acid cytoplasmic domain would be helpful in analyzing the properties of CR2–p53 binding.

Our data demonstrate that polyclonal anti-idiotypic antibodies prepared against solubilized and purified receptors can be helpful in the analysis of their conformational domains and in the identification of their specific ligands, especially those binding with low affinity.

p53 recognized by Ab2 and reactive with CR2 was identified as the p53 antioncogene-encoded intracellular protein on the basis of (*i*) its recognition by Pab1801, an anti-p53 mAb (26); (*ii*) its expression in various transformed cell lines and its absence in normal cells (27); (*iii*) detection by Ab2 of a 53/55-kDa doublet from U-937 cells, as also described in some other transformed cells (32); (*iv*) identical isoelectric points of 7.2 (32); (*v*) nuclear and plasma membrane localization of p53 (33); and (*vi*) *in vitro* phosphorylation in the presence of $MnCl_2$ (34).

We showed previously (11) that phosphorylation of membrane or nuclear CR2 could be induced in the presence of $MgCl_2$ but was highly enhanced in the presence of $MnCl_2$. CR2 was phosphorylated not only on tyrosine but also on serine residues. p53 was phosphorylated on serine residues (34). These data suggest that an identical serine kinase could be involved, at the membrane level, in the phosphorylation of CR2 and p53.

The presence of p53 in CR2-positive as well as CR2-negative human transformed cells is in accord with the ability of the p53 antioncogene-encoded phosphoprotein to react with other intracellular molecules. Indeed, p53 has been shown to form complexes with viral nuclear oncoproteins, including EBV nuclear antigen (35), simian virus 40 large tumor antigen (36), and adenovirus 55-kDa E1B protein (37), and with a cellular heat shock protein, hsp 72–73 (38). p53 was inactivated when bound to simian virus 40 large tumor antigen and adenovirus 55-kDa E1B protein. The gene encoding p53 was originally identified as an oncogene (25) because it could immortalize primary rodent cells by itself and could cooperate with *ras* for transformation. Recently, Hinds *et al.* (39) showed that the wild-type p53 gene failed to cooperate with *ras* to transform primary cells. It was suggested that activation of p53 transforming activity was due to inactivation of the wild-type p53 activity (40). Thus, the p53 protein appeared to be a regulatory antioncogene protein which reacted with oncogene proteins.

Therefore, our evidence that CR2 can associate with p53, an antioncogene-encoded phosphoprotein, suggests that CR2, which can act as a growth factor receptor (41) and a virus receptor (2) and is expressed at a "narrow window" of the B-cell differentiation pathway (42), has properties similar to those of oncogene-encoded proteins.

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