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Unidirectional Potentiation of Binding Between Two Anti-FBP MAbs: Evaluation of the Involved Mechanisms

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Abstract The monoclonal antibody MOv19 directed to a folate binding protein shows temperature-dependent potentiation of binding of the noncompeting monoclonal antibody MOv18 to the relevant antigen, but the mechanism involved in this phenomenon had remained unclear. Use of chimeric versions of both monoclonal antibodies and the $F(ab')_2$ and Fab fragments of MOv19 revealed an increment in MOv18 binding in all combinations irrespective of the origin of the Fc portion of the monoclonal antibody. The potentiating effect of bivalent MOv19 fragments on ^{125}I-MOv18 binding was similar to that of the entire monoclonal antibody and occurred at saturating concentrations of both reagents at which monovalent binding prevails. Similarly, the monovalent fragment also induced a significant increase in MOv18 binding. However, the potentiation occurred only at very high concentrations of antibody fragment. Homologous inhibition was drastically reduced using MOv19 Fab fragment, suggesting a low binding stability of the monovalent reagent. Immunoblotting analysis and binding in the presence of exogenous purified folate binding protein indicated a cross-linking between soluble and cell surface molecules mediated by the bivalent monoclonal antibodies. The extent of the increase in MOv18 binding at 0°C with high amounts of exogenous folate binding protein was lower than that obtained at 37°C in the absence of added molecule. Release of ^{125}I-MOv18 from the cell surface was significantly higher in the absence of MOv19 than in its presence. Affinity constant values of ^{125}I-MOv18 binding evaluated in the presence of MOv19 or control monoclonal antibody MINT5 were comparable, whereas the number of binding sites per cell detected by ^{125}I-MOv18 was significantly higher in the presence of MOv19 than MINT5. Together, the data suggest that monoclonal antibody MOv19 induces a conformational change of the molecule it binds that increases the number of antigenic sites available for MOv18 binding and, in turn, the binding stability of the latter. MOv19 bivalency also contributes to the MOv18 binding increment by cross-linking released and cell surface–anchored folate binding protein molecules.

Key words: monoclonal antibodies, folate binding protein, human ovary carcinoma, conformational change, potentiation

The application of monoclonal antibody (MAbs) to the treatment of cancer has proven to be more difficult than initially anticipated. Binding of these reagents to the target antigen is limited, in part, by the specificity, accessibility, and local density of the antigen [Colnaghi et al., 1993]. Several studies have demonstrated enhanced labeling of tumour cells in vitro and tumour targeting in vivo by using a combination of rather than single MAbs directed to different antigens [Mujoo et al., 1991; Yu et al., 1990]. Combinations of noncompeting MAbs directed to the same antigen have also been reported to enhance antibody-dependent cell-mediated cytotoxicity [Fogler et al., 1988], to potentiate cytotoxicity of immunotoxin [Byers et al., 1988] in vitro, and to exert a synergistic antiproliferative effect [White et al., 1990; Kasprzyk et al., 1992] in vivo.

Some of these approaches to antibody-driven therapy involve receptors for nutrients or growth factors as target molecules [Harris, 1990]. Among the nutrient receptors, the folate binding protein (FBP) is homogeneously overexpressed in almost all nonmucinous ovary carcinomas [Boerman et al., 1991; Coney et al., 1991]. Two distinct FBP forms have been isolated: a membrane form, which is anchored to the plasma membrane by a fatty acid linkage, identified as a glycosylphosphatydilinositol (GPI) tail [Miotti

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et al., 1992], and a soluble form which has been detected in the spent medium of carcinoma cell lines as well as in some biological fluids [Tomassetti et al., 1993; Mantovani et al., 1994].

Our previous analysis using paired combinations of three noncompeting MAbs (MOv17, MOv18, and MOv19) directed to the FBP molecule revealed a temperature-dependent increment of ^{125}I-labeled MOv18 binding in the presence of unlabeled MOv19 [Casalini et al., 1991]. In the present work, we have investigated the mechanism responsible for this phenomenon. Our data suggest a model in which the binding of MOv19 induces a conformational change in the FBP resulting in greater accessibility to the epitope recognised by MOv18 and a greater stability of MOv18 binding.

MATERIALS AND METHODS

Tumor Cell Lines

The human ovarian carcinoma cell lines OVCA432 and IGROV1 were kindly provided by Dr. R. Knapp (Dana Farber Institute, Boston, MA) and Dr. J. Bénard (Institute Gustave Roussy, Villejuif, France), respectively, and were maintained in RPMI-1640 medium (Microbiological Associates, Walkersville, MD) supplemented with 10% fetal calf serum (FCS), penicillin (100 μg/ml), and streptomycin (100 μg/ml). Both cell lines overexpress FBP [Miotti et al., 1992], whereas only IGROV1 cells express the epidermal growth factor receptor (EGFR) [Ferrini et al., 1993].

Monoclonal Antibodies

MAbs MOv17 (IgG_1), MOv18 (IgG_1), and MOv19 (IgG_{2a}), raised against human ovarian carcinoma and directed to FBP [Miotti et al., 1987; Casalini et al., 1991], and MAb MINT5 (IgG_1) raised against EGFR [Gadina et al., 1993], were used as mouse ascites purified by affinity chromatography on protein A–Sepharose Cl-4B (Pharmacia Biotech Europe, Brussels, Belgium). Chimeric MOv18 (Chi-MOv18) and MOv19 (Chi-MOv19) [Coney et al., 1994] were kindly provided in purified form by Dr. L. Coney (Apollon, Malvern, PA). These chimeric MAbs have the same human IgG_1 constant region [Oi et al., 1986].

Purified MAbs were labeled by lactoperoxidase-catalyzed iodination [Casalini et al., 1991] to a mean specific activity of 9 μCi/μg.

MOv19 $F(ab')_2$ fragments were obtained by pepsin digestion (ratio of pepsin:MAb = 4:100) in sodium acetate buffer, pH 4.2, for 6 h at 37°C. The reaction was stopped by the addition of ImmunoPure IgG purification binding buffer (1:2 dilution) (Pierce, Rockford, IL), and the sample was loaded onto a protein A–AffinityPack column (Pierce) to remove undigested MAb. The eluate was dialysed against phosphate-buffered saline (PBS) using dialysing tubing with a molecular weight cutoff of 50,000. MOv19 Fab fragments were obtained using the ImmunoPure Fab preparation kit (Pierce).

SDS-polyacrylamide gel was run under nonreducing conditions using Coomassie blue staining essentially as described [Laemmli, 1970]. The detection of a single band at Mr 110,000 and at Mr 48,000 for $F(ab')_2$ and Fab, respectively, indicated >98% purity of the preparations.

Potentiation of ^{125}I-MOv18 Binding

Live cells (30,000 cells/well) were incubated in 96-well U-bottom plates (Dynatech Laboratories, Inc., Chantilly, VA) in RPMI-1640–1% FCS for 3 h at either 37 or 0°C with 50 μl of fixed (1.5 μg/ml) or titrated doses of unlabeled MOv19 or MOv19 fragments (starting from 150 μg/ml for MAbs and $F(ab')_2$ or 675 μg/ml for Fab) in the presence of a fixed amount of ^{125}I-MOv18 (0.6 μg/ml, 500,000 cpm/well). After centrifugation, pellets were washed three times with PBS–0.03% bovine serum albumin (washing buffer), and bound radioactivity was determined.

Immunoblotting Assay

Live cells (2×10^5) were incubated with 50 μl of different dilutions of unlabeled MOv19 in RPMI-1640–1% FCS for 15 min at 37°C. A trace amount of MOv18 (10 ng/ml) was added, and incubation continued for an additional 3 h. After centrifugation, pellets were washed twice with washing buffer and incubated for 20 min at room temperature with 50 mM glycine-HCl, 100 mM NaCl, pH 2.8. After adjusting pH with 1 M Tris-HCl, pH 9, supernatants were run under nonreducing conditions in a 10% slab SDS-polyacrylamide gel. The gel was transferred to Hybond-C Super nitrocellulose (Amersham, Little Chalfont, UK) as described [Towbin et al., 1979]. The immunoblot analysis was performed using an enhanced chemiluminescence (ECL) Western blot detection system (Amersham) essentially as described by the manufacturer. Immunoreaction was carried out with 10 μg/ml of

anti-FBP rabbit serum [Coney et al., 1991] and biotinylated antirabbit IgG.

Release of ^{125}I-MAb From the Cell Surface

Live cells (2×10^5) were incubated in complete culture medium for 1 h at 0°C with ^{125}I-MOv18 (0.6 ng/ml, 20,000 cpm/200 μl) alone or in the presence of unlabeled MOv19 (1.5 μg/ml). Cells were washed twice and incubated at 37°C for different times in the same medium; both the radioactivity bound to the cells and that present in the supernatants were assessed. Supernatants were then TCA-precipitated (20% final concentration) overnight at −20°C and centrifuged for 15 min at 6,000*g*. Pellets accounted for >95% of the radioactivity in all samples.

Evaluation of K_{aff} and Number of Antigenic Sites

Cells (30,000 live cells/well, two replicates) were incubated in the presence of a fixed amount of unlabeled MAb (7.5 μg/ml in RPMI-1640–1% FCS) for 1 h at 37°C and further incubated for 3 h on ice with serial dilutions of ^{125}I-MOv18 starting from 3×10^6 cpm/well (1.8 μg/ml). After three washes with cold washing buffer, cell pellets were assessed for radioactivity in a gamma counter. ^{125}I-MOv18 nonspecific binding was evaluated as bound radioactivity in the presence of a 100-fold excess of unlabeled MOv18.

Scatchard analysis of the binding data was elaborated on a desktop computer with a program calculating the molarity of the bound antibody, the ratio of bound/free ligand molecules, the K_{aff}, and the number of binding sites per cell.

DDIRMA MOv18/^{125}MOv19

Purified MOv18, adsorbed on polystyrene beads, was used as catcher antibody, while ^{125}I-MOv19 was used as tracer [Miotti et al., 1992]. Cell supernatants were diluted 1:2 with 200,000 cpm ^{125}I-MOv19 in PBS–10% FCS and incubated overnight at room temperature with MOv18-coated beads. Beads were washed with PBS and counted for radioactivity. Data are given as binding index—that is, the ratio of cpm bound in the presence of cell supernatants to cpm bound in the presence of medium alone (~500 cpm). binding index (B.I.) ≥3 was considered positive.

Statistical Analyses

Mean values were compared by two-sided Student's *t*-test. In the case of the Scatchard plots, the curve was evaluated by linear regression analysis and the slopes of the regression lines compared by the test of parallelism.

RESULTS

In all experiments, the increment in MOv18 binding induced by the presence of MOv19 was analysed on the ovarian carcinoma cell lines OVCA432 and IGROV1. The anti-FBP MOv17 or the anti-EGFR MINT5 MAb served as control. The control at 0°C confirmed the absence of an increase in MOv18 binding in the presence of MOv19.

Relevance of MAb Fc Portion and MAb Valency

Figure 1 shows results of a representative experiment to analyse potentiation of MOv18 binding by MOv19 using chimeric versions of each MAb. The maximum increment in MOv18 binding was found at saturating concentrations of MOv19, and no prozone phenomenon was observed. A similar binding increment was evident in all combinations of murine and chimeric MAbs tested, irrespective of the origin of the Fc portion of MOv19.

MOv19 F(ab')$_2$ fragments competed the binding of the intact homologous radiolabeled MAb at both 37°C and at 0°C with an efficiency equal to or even greater than that of the entire MAb (Table I). By contrast, MOv19 Fab showed very low competing activity, especially at 37°C, and inhibition levels were comparable to those of the entire molecule only when Fab was used at a

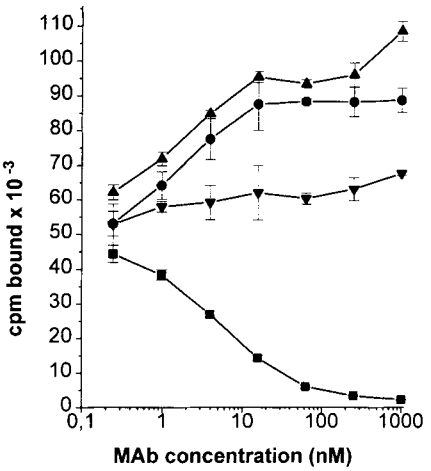

Fig. 1. Evaluation of potentiating effect of MOv19 on ^{125}I-Chi-MOv18 binding on IGROV1 cells. Cells were incubated with various concentrations of MAbs: (●) murine MOv19, (▲) Chi-MOv19, (■) Chi-MOv18, and (▼) murine MINT5. Error bars represent SE of three replicates.

200-fold higher concentration. The enhancement of ^{125}I-MOv18 binding by MOv19 bivalent fragments was similar to that of the entire MAb. The increment was detected at MOv19 saturating concentrations, at which monovalent binding predominated. The monovalent MOv19 Fab fragments increased binding only at very high concentrations (250-fold higher than concentrations of entire Mab) (Table I).

Experiments to evaluate the potentiating effect of MOv19 on ^{125}I-MOv18 binding at 37°C to two different target cells indicated that MOv19 Fab consistently increased ^{125}I-MOv18 binding, although the mean increment of binding was lower than that obtained in the presence of whole MAb (47% vs. 87% on OVCA432 and 41% vs. 64% on IGROV1) (Table II). Although the purity of the Fab was greater than 98%, we cannot exclude the possibility that 1 or 2% of contaminating whole antibody accounts for the observed increase in ^{125}I-MOv18 binding.

Relevance of Soluble Antigen

Cells were incubated with MOv18 in the presence of MOv19, washed, and treated at pH 2.8 for antigen-antibody dissociation and the supernatants analysed for the presence of the relevant antigen. Since this pH does not induce cell surface antigen release [Miotti et al., 1992], FBP should be detected in the supernatant only when bivalent MAb cross-link to the cell surface. In repeated experiments, immunoblotting analysis of pH 2.8 supernatants revealed that soluble FBP was maintained at the cell surface by MOv19 in a dose-dependent way, whereas no soluble molecule (gp38) was detectable with MOv18 or medium alone; Figure 2 shows the results obtained with OVCA432 cells.

TABLE I. Binding Activity of MOv19 and Its Fragments on OVCA432 Cells

MAb	Incubation temperature (°C)	MAb concentration (nM) needed to Compete[a] ^{125}I-MOv19	Potentiate[b] ^{125}I-MOv18
MOv19	0	6.0	—[c]
	37	5.0	50.0
MOv19 F(ab')$_2$	0	1.9	—
	37	2.0	40.0
MOv19 Fab	0	35.0	—
	37	900.0	13,500.0

[a]Data are extrapolated from dose-response curves of a simultaneous experiment and are reported as the dose required to compete 50% of binding.
[b]Data are extrapolated from dose-response curves of a simultaneous experiment and are reported as the dose required to give maximum binding increment.
[c]Binding increment <30% at the maximum concentration tested.

TABLE II. Potentiating Effect of MOv19 on ^{125}I-MOv18 Binding to Two Different Cell Lines

Target	Added cold MAb	Binding increment[a]	Number of experiments
OVCA432	MOv19	87.3 ± 11.8	5
	MOv19 Fab	47.25 ± 4.8	4
	MOv17	12.8 ± 5.8	5
	MINT5	12.4 ± 7.0	5
IGROV1	MOv19	63.7 ± 26.0	3
	MOv19 Fab	41.5 ± 10.5	2
	MOv17	10.3 ± 6.0	3
	MINT5	17.3 ± 13.8	3

[a]Mean percent ± SE. Data refer to concentrations of cold MAb corresponding closely to those reported in Table I. Experiments were carried out at 37°C.

^{125}I-MOv18 binding was evaluated at 0°C, at which no potentiating effect of MOv19 is observed [Casalini et al., 1991], in the presence or absence of exogenously added purified FBP (gp38) [Tomassetti et al., 1993] and compared to the binding observed in standard conditions (37°C with no exogenous added FBP). DDIRMA analysis was performed to quantitate soluble FBP (spontaneously released and experimentally added) in the cell supernatants. Figure 3 shows that in the absence of FBP no spontaneously released antigen was detected by DDIRMA at 37°C or at 0°C, whereas the increment of MOv18 binding at 37°C was consistent (82% and 44% in OVCA432 and IGROV1, respectively). A binding increment was detected at 0°C in the presence of exogenous purified FBP (up to 40×10^{-6} nmoles) but never exceeded 32% in OVCA432 and 8% in IGROV1 cells. Thus, the presence of soluble FBP cannot completely account for the binding increase.

Effect of MOv19 on MOv18 Binding

^{125}I-MOv18 release from the cell surface was evaluated in the presence or absence of unlabeled MOv19. After MAb binding at 0°C, cells were incubated at 37°C for different time periods. In both cell lines, and at every time point (with the exception of IGROV1 at 120 min), the radioactivity detected in the supernatants was significantly higher when ^{125}I-MOv18 was tested alone than in the presence of MOv19 (Fig. 4),

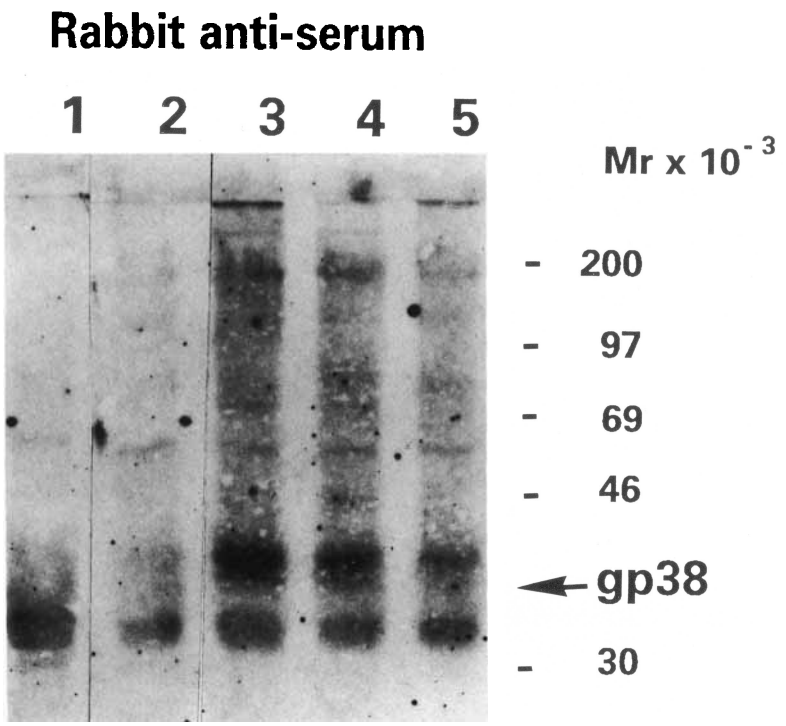

Fig. 2. Immunoblot detection of FBP (gp 38) in the pH 2.8 supernatant from OVCA432 cells. Before acid treatment, cells were incubated with (lane 1) medium alone, (lane 2) MOv18, (lane 3) MOv18 in the presence of 1.6 μg/ml unlabeled MOv19, (lane 4) MOv18 in the presence of 0.4 μg/ml MOv19, or (lane 5) MOv18 in the presence of 0.1 μg/ml MOv19.

whereas the presence of the control MAb MINT5 did not affect ^{125}I-MOv18 release (data not shown).

Scatchard analysis revealed comparable ^{125}I-MOv18 K_{aff} values evaluated in the presence of MOv19 or the control MAb MINT5, whereas the number of binding sites per cell detected by ^{125}I-MOv18 was higher in the presence of MOv19 than MINT5 (Table III). Two independent experiments revealed a reproducible and significant increase of detectable binding sites in both OVCA432 and IGROV1 cells (in two independent experiments, the percent increase was 32.2 and 23.4% in OVCA432 cells and 14.8 and 13.2% in IGROV1 cells). Figure 5 shows the binding data and the Scatchard analysis of one of the two experiments.

DISCUSSION

The present data suggest that the increment in MOv18 binding in the presence of MOv19 reflects the induction of a conformational change in the FBP upon MOv19 binding. Such a conformational change increases the number of available MOv18 binding sites and the binding stability of MOv18. MOv19 bivalency also contributes to the MOv18 binding increment by cross-linking between released and cell surface–anchored FBP molecules.

Ehrlich et al. [1982] initially proposed an Fc interaction between two antibodies to explain the observed enhanced affinity on human chorionic gonadotropin. Later, Moyle et al. [1983] demonstrated that a circular antigen-antibody complex was responsible for the increased affinity, since $F(ab')_2$ but not Fab fragments increased binding affinity. More recently intermolecular cooperativity between Fc portions of IgG_3 molecules was proposed as a relevant contribution to the strength of antibody binding to multivalent antigens [Greenspan et al., 1992]. In our study, the binding increase was independent of Fc interactions since chimeric MAbs had essentially the same effect as murine MAbs, and the phenomenon was also evident using MOv19 $F(ab')_2$ and, albeit to a lesser extent, with MOv19 Fab fragment.

The results of immunoblotting analysis indicating that MOv19 anchors soluble FBP to the cell membrane are consistent with the notion of

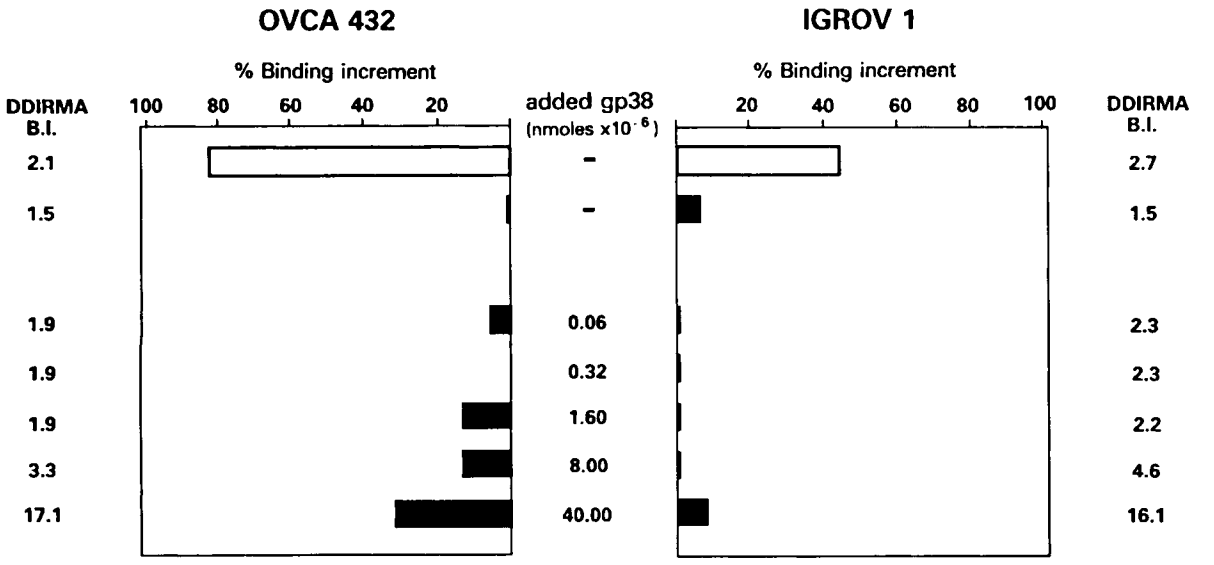

Fig. 3. Evaluation of ^{125}I-MOv18 binding increment in the presence or absence of purified added FBP (gp 38) in OVCA432 and IGROV1 target cells. Experiments were performed either at 37°C (□) or 0°C (■). The percent increase of ^{125}I-MOv18 binding was evaluated in the presence of a fixed amount of MOv19 (5×10^{-4} nmoles) and various dilutions of purified FBP starting from 40×10^{-6} nmoles. The MOv19-MOv18 DDIRMA B.I. values of the corresponding supernatants are given.

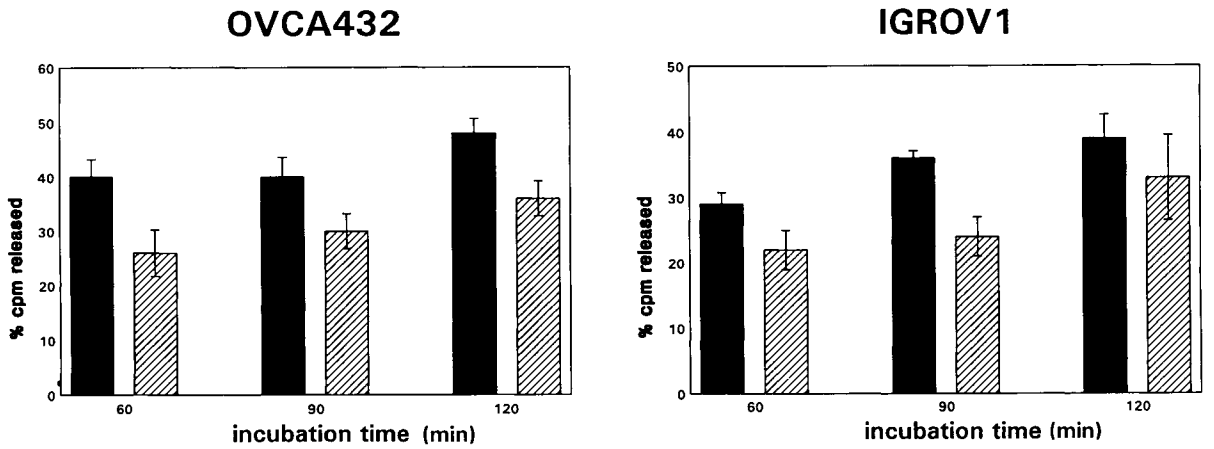

Fig. 4. Time course of release of ^{125}I-MOv18 bound to target cells into the supernatants. Cells were incubated with ^{125}I-MOv18 alone (solid bar) or in the presence of unlabeled MOv19 (hatched bar). Error bars represent SE of 3–5 experiments. The release of ^{125}I-MOv18 was significantly reduced by the presence of unlabeled saturating concentrations of MOv19 at every time point in both target cells, with the exception of IGROV1 at 120 min ($P < 0.01$ by two-sided Student's t-test).

a local increase of available MOv18 epitopes due to MOv19 MAb cross-linking. The experiments in which soluble FBP was added indicated the requirement for much larger amounts of soluble molecule than those spontaneously released in order to obtain a binding increment similar to that detected in standard conditions at 37°C. However, we cannot exclude the possibility that the cell membrane microenvironment is more favourable to antigen-antibody interactions. On the other hand, no binding increment was evident with the reverse MAbs combination (i.e., ^{125}I-MOv19 and cold MOv18 [Casalini et al., 1991]) and addition of purified FBP did not increase ^{125}I-MOv19 binding (data not shown). This unidirectional phenomenon suggests that steric hindrance due to the position of the MOv18-recognised epitope on the molecule might prevent cross-linking between two FBP molecules by MOv18. According to this hypothesis,

TABLE III. Scatchard Plot Analysis of ^{125}I-MOv18 Binding in the Presence of Unlabeled MAbs on Two Different Cell Lines*

MAb mixture	K_{aff} (1/nM)	Antigenic sites No./cell ($\times 10^5$)	Increment[a]
OVCA432			
MOv18 + MOv19	995	6.32	28
MOv18 + MINT5	948	4.93	—
IGROV1			
MOv18 + MOv19	616	30.27	14
MOv18 + MINT5	614	26.49	—

*Data are reported as mean of two different experiments.
[a]Percent increment of MOv18 binding sites in the presence of MOv19 over those detected in the presence of the control MAb MINT5.

all FBP molecules expressed on the cell membrane are available for MOv19 binding, but only some are available for MOv18. Experimental support for such a model comes from the observation that the number of antigenic sites detected by MOv19 is at least 30% higher than that recognised by MOv18 [Miotti et al., 1987; Coney et al., 1994].

In tracer conditions (i.e., at antigen excess), controversial results about MAb monovalent or bivalent binding have been reported [Mason et al., 1980; Dower et al., 1984]. By contrast, there is general agreement that monovalent binding prevails when the MAbs are tested at saturating concentrations. In all of the present experiments, MOv19 was present in molar excess with respect to the cell surface FBP molecules, so that its interaction is expected to be mainly monovalent.

The evidence that the MOv18 binding increment was mediated in conditions in which a monovalent binding predominates argues for a conformational change induced by MOv19. Accordingly, monovalent MOv19 Fab fragment also gave a significant increment in MOv18 binding, although only at very high concentrations, probably reflecting the low binding stability of the monovalent reagent. Indeed, a drastic reduction in homologous inhibiting capability was observed using MOv19 Fab fragment at 37°C compared to that at 0°C. When the Fab_2 and Fab fragments were tested at optimal concentrations, the former reagent gave consistently higher binding increments. It is likely that MOv19 bivalency contributes to the MOv18 binding increment by cross-linking between released and cell surface–anchored FBP molecules.

A conformational change in a molecule might result in increased binding affinity [Byers et al., 1988] or in easier accessibility to additional binding sites [Saito et al., 1991]. On the basis of the MAb release experiments, an increase of either affinity or accessible sites might be expected. Scatchard analysis of MOv18 binding revealed no change in K_{aff} and an increase in the number of binding sites. The particular experimental conditions used (i.e., incubation at 37°C with a saturating concentration of cold MAb) might account for the lower K_{aff} as compared to previously reported values [Miotti et al., 1987]. Moreover, the second incubation at 0°C could partially reverse the FBP conformational change and account for the relatively modest binding increment of ^{125}I-MOv18. Although modest, the increment was reproducible in two independent experiments on two different target cells. Moreover, the parallelism of the linear regression in the presence of MOv19 or of control MAbs argues for the significance of the increase in MOv18 binding sites. The monophasic equilibrium binding kinetics indicated that the new accessible MOv18 binding sites belong to the same class as the previously available ones.

The FBP conformational change might rest largely in the topobiology of this molecule, which is anchored through a GPI tail [Miotti et al., 1992] and therefore is particularly flexible on the cell membrane at 37°C. Moreover, the FBP clustering in caveolae during folate influx [Anderson et al., 1992] facilitates a local increase in antigen concentration.

MOv18 MAb, directed against the FBP, has been used successfully in a clinical setting both for radioimaging and radioimmunotherapy [Crippa et al., 1991; Crippa, 1993]. Furthermore, bispecific MAbs capable of selective binding to FBP on tumour cells and to a lymphocyte activation molecule have been developed as immunotherapeutic agents [Canevari et al., 1992]. A Phase II clinical trial with bispecific $F(ab')_2$ MOv18/anti-CD3 [Bolhuis et al., 1992] for intraperitoneal treatment of ovarian carcinomas is now in progress.

The increase in MOv18 binding in the presence of MOv19 was observed in vitro independent of the form of the MAb used. Although the in vivo relevance of the phenomenon remains to be demonstrated, preliminary preclinical experiments in athymic mice bearing IGROV1 xenotransplants indicate that a higher MOv18 radiolocalization occurs in the presence of MOv19

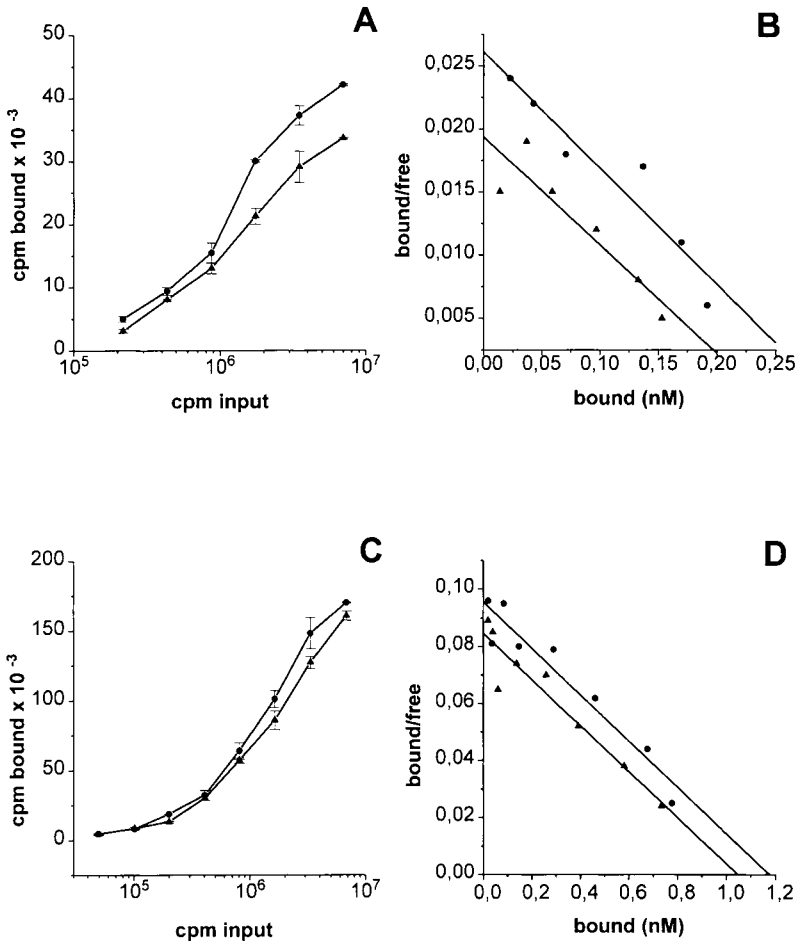

Fig. 5. ^{125}I-MOv18 binding in the presence of unlabeled MOv19 (●) or MINT5 (▲) on OVCA432 (**A,B**) and IGROV1 (**C,D**) cells. A,C: Binding plots. B,D: Respective Scatchard plots. Statistical analysis of the curves: on OVCA432: ^{125}I-MOv18 + MOv19, $R^2 = 0.8917$, ($P < 0.0046$). ^{125}I-MOv18 + MINT5, $R^2 = 0.8126$ ($P < 0.0141$); on IGROV1: ^{125}I-MOv18 + MOv19, $R^2 = 0.9237$ ($P < 0.0001$). ^{125}I-MOv18 + MINT5, $R^2 = 0.9117$ ($P < 0.0002$). In both cell lines, the test of parallelism indicated that the regression lines in the presence of MOv19 were parallel to those in the presence of MINT5. The percent increment of MOv18 binding sites in the presence of MOv19 over those detected in the presence of MINT5 was 32.2 on OVCA432 and 13.2 in IGROV1 cells.

(data not shown). Clinical studies are needed to determine whether the increment in MOv18 binding by MOv19 can be exploited for immunotherapy or immunodiagnostic strategies.

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