

# Distribution of OV-TL 3 and MOv18 in normal and malignant ovarian tissue

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## Abstract

**Aims**—To analyse the distribution of OV-TL 3 and MOv18 in normal ovarian tissue to determine which antibody is most suitable for (radio)immunotherapy of ovarian carcinoma.

**Methods**—The distribution of OV-TL 3 and MOv18 was determined using immunohistochemistry and flow cytometry.

**Results**—Epithelial and other cells in many tissues, and leucocytes in peripheral blood, bone marrow and spleen stained positively with OV-TL 3. The staining pattern of MOv18 in normal tissues was more restricted and was confined to epithelial cells in the lung, kidney, pancreas, salivary gland, ovary, Fallopian tubes, and cervix. Reactivity was also observed with pneumocytes in the lung, tubuli in the kidney, acinar cells in the salivary gland and pancreas, in the placenta, and with Kupffer cells in the liver. The staining pattern of chimeric MOv18 was identical with the murine form. OV-TL 3 and MOv18 reacted with 100% and 98% (45/46) of the 46 tested epithelial ovarian cancers, respectively. In ovarian carcinoma tissue homogeneous staining of epithelial cells was observed with OV-TL 3 and more heterogeneous staining with MOv18. In 12 and nine patients, respectively, a difference in staining intensity for OV-TL 3 and MOv18 was observed between various tumour samples from the same patient.

**Conclusion**—MOv18 has greater therapeutic potential because of its restricted reactivity with normal tissues and especially, in contrast to OV-TL 3, its lack of reactivity with haematopoietic cells.

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Keywords: Ovarian carcinoma, antigen expression, targeting.

Tumour associated antigens are promising targets for monoclonal antibodies in immunoscintigraphy and immunotherapy. OV-TL 3 and MOv18 are both directed against antigens highly expressed on ovarian carcinoma cells.<sup>1,2</sup> Both of these monoclonal antibodies have been applied in pre-clinical and clinical immunotargeting studies.<sup>3-7</sup> The murine monoclonal antibody MOv18 (m-MOv18) has been chimerised by fusing the murine variable part with the human constant IgG1 region to reduce immunogenicity.<sup>8</sup> Chimeric MOv18 (c-MOv18) has been studied in a xenograft murine model and in patients suspected of having

ovarian carcinoma.<sup>7,9</sup> The antigen recognised by OV-TL 3, OA3, was identified as a novel multimembrane spanning protein,<sup>10</sup> whereas the antigen recognised by MOv18 is a membrane associated, folate binding protein (FBP).<sup>11,12</sup> In monoclonal antibody based immunotherapy in which the antibody is used alone, or conjugated to toxins, drugs or radio-nuclides, the reactivity with normal tissues has to be limited to avoid non-specific targeting of normal tissues. Staining with OV-TL 3 was reported to be negative in normal tissues, except for weak and irregular reactivity in some ovarian cysts and in epithelial cells lining the female genital tract.<sup>1</sup> Normal bone marrow and blood cells were not studied. In the initial report MOv18 showed no reactivity with normal tissues,<sup>2</sup> but Stein *et al* reported reactivity with the epithelium of renal tubules, pancreas, lung, salivary glands, Fallopian tubes, and endocervix.<sup>13</sup>

In the present study we have analysed the normal tissue distribution of OV-TL 3, m-MOv18 and c-MOv18 more extensively to select the most appropriate antibody for (radio)immunotherapy of ovarian carcinoma. Primary tumours and metastases of 46 patients with ovarian carcinoma were also examined using immunohistochemistry.

## Methods

### MONOCLONAL ANTIBODIES

OV-TL 3 is a murine IgG1 monoclonal antibody that reacts with an antigen which is highly expressed on 90% of ovarian carcinomas of different histological types.<sup>1,14-16</sup> MOv18 is also of the IgG1 subclass and the antigenic determinants recognised by MOv18 are abundantly present on the cell surface of ovarian carcinoma cells.<sup>2</sup> Monoclonal antibody OC 125, which recognises the ovarian carcinoma associated CA125 antigen, was used as a control antibody for the intensity of staining of ovarian carcinoma tissues.<sup>17</sup> OV-TL 3, m-MOv18, c-MOv18, and OC 125 were kindly provided by Dr S O Warnaar (Centocor, Leiden, The Netherlands). MOPC-21 (IgG1; Organon Teknika, Boxtel, The Netherlands) or anti-KLH (IgG1; Becton Dickinson, Erembodegem, Belgium), both directed against non-human antigens, were used as negative controls.

### TISSUE

Normal human tissues were obtained at surgery and snap frozen in liquid nitrogen. Sections 6 µm thick were cut on a cryostat, fixed with

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acetone for 10 minutes at room temperature, air dried and stored at  $-80^{\circ}\text{C}$  until analysis. Normal tissues obtained from different individuals were tested. In addition, 156 biopsy specimens of ovarian carcinoma, taken from different locations, were obtained at surgery from 46 patients who underwent laparotomy for ovarian carcinoma. Eighteen patients underwent primary debulking surgery and 28 underwent laparotomy after initial surgery and chemotherapy for ovarian carcinoma. All but one patient had advanced ovarian carcinoma at the time of surgery. One patient with primary ovarian carcinoma had FIGO (Fédération Internationale de Gynaecologie et Obstetrique) stage Ic disease. If present, tissue was obtained from the malignant ovary, as well as from peritoneal metastases. All tissues were processed as described for normal tissues. Samples were assessed histopathologically for determination of the histological type and differentiation grade. Of the 46 ovarian carcinomas, 40 were serous, three were mucinous, two were endometrioid ovarian carcinomas, and one tumour was of the clear cell type.

#### IMMUNOHISTOCHEMISTRY

Tissue sections were washed in phosphate buffered saline (PBS) and incubated with the monoclonal antibody for 60 minutes at room temperature. After washing, the sections were incubated with peroxidase conjugated rabbit antimouse immunoglobulin (ITK Diagnostics, Uithoorn, The Netherlands) for 30 minutes. The staining reaction was developed by incubating the sections for five to 10 minutes

with 0.05% (w/v) 3'-3'-diaminobenzidine tetrahydrochloride (DAB) in PBS, containing 0.02% hydrogen peroxide (Sigma, St Louis, Missouri, USA). The sections were washed in PBS, counterstained with haematoxylin and mounted in Depex. Various concentrations of purified OV-TL 3, m-MOv18 and c-MOv18 were studied on control sections of an ovarian carcinoma (2, 10, 20, 50, and 100  $\mu\text{g/ml}$ ). The optimal concentration was determined to be 50  $\mu\text{g/ml}$  for purified m- and c-MOv18 and 20  $\mu\text{g/ml}$  for purified OV-TL 3. In each assay serial sections of an ovarian carcinoma, known to react with both antibodies, were tested simultaneously. The intensity of the staining reaction, relative to positive staining of OC 125 on malignant ovarian tissues, was scored as follows: strongly positive (++) ; moderately positive (+) ; faint/weak ( $\pm$ ) ; or negative (-). The extent of staining was also defined as either homogeneous (>90% of the epithelial cells positive), heterogeneous (50-90% positive), or focal staining (10-50% positive). All sections were analysed by two observers.

#### FLOW CYTOMETRY

Fresh peripheral blood was obtained from apparently healthy individuals and bone marrow aspirates from patients without carcinoma. Spleen cells were obtained by homogenising a spleen through a tissue sieve. White blood cells from peripheral blood and bone marrow were prepared by Ficoll-Hypaque gradient centrifugation (Pharmacia, Uppsala, Sweden) and extensively washed in 0.1% bovine serum albumin (BSA) in PBS (PBS/BSA).

Table 1 Normal tissue reactivity of OV-TL 3 and MOv18<sup>a</sup>

Tissue	No. of specimens tested	Cell type	OV-TL 3 staining	Cell type	MOv18 staining <sup>b</sup>
Brain	2	White cortex	+ <sup>c</sup>		-
Peripheral nerve	3	Perineurium	+		-
Skin	10	Endothelial cells	+		-
		Sebaceous glands	+		$\pm$
		Hair follicles	$\pm$		-
			-		-
Striated muscle	10		-		-
Peritoneum	10		-		-
Spleen	3	Red pulp	+ <sup>c</sup>		-
Lymph node	10		$\pm$		-
Tonsil	3	Lymphocytes	$\pm$ <sup>c</sup>		-
Thymus	3	Lymphoid tissue	$\pm$	Stromal cells	$\pm$
Heart	3		-		-
Lung	3	Bronchial epithelium	+		+
		Pneumocytes	-		$\pm$
		Serous acini	$\pm$		$\pm$
Salivary gland	3	Collecting ducts	$\pm$		$\pm$
			-		-
Stomach	3		-		-
Colon	5	Goblet cells	+		-
		Ganglion cells	+		-
		Ductal epithelium	$\pm$		+
		Acini	-		+
		Langerhans' cells	$\pm$		-
Liver	5	Kupffer cells	$\pm$		$\pm$
		Glomeruli	+		-
Kidney	5	(Bowman's capsule)		Distal tubuli	+
		Transitional cells	$\pm$		-
Bladder	2		-		-
Thyroid	3		-		-
Adrenal gland	2		-		-
Breast	3	Myoepithelium	$\pm$		-
		Lobuli	$\pm$		-
		Follicular epithelium	+		+
Ovary	10	Epithelial lining	+		+
Fallopian tube	5		+		+
Uterus	5		-		-
Cervix	5	Epithelium	$\pm$		$\pm$
Placenta	1	Trophoblast	+		+
Testis	2	Rete testis	$\pm$		-
Prostate	2	Epithelium	+		-

<sup>a</sup> Intensity of staining: ++, strongly positive; +, moderately positive;  $\pm$ , weak/faint; -, no staining. <sup>b</sup> The staining pattern for m- and c-MOv18 was identical. <sup>c</sup> Cell type not evaluable because of very high background staining.

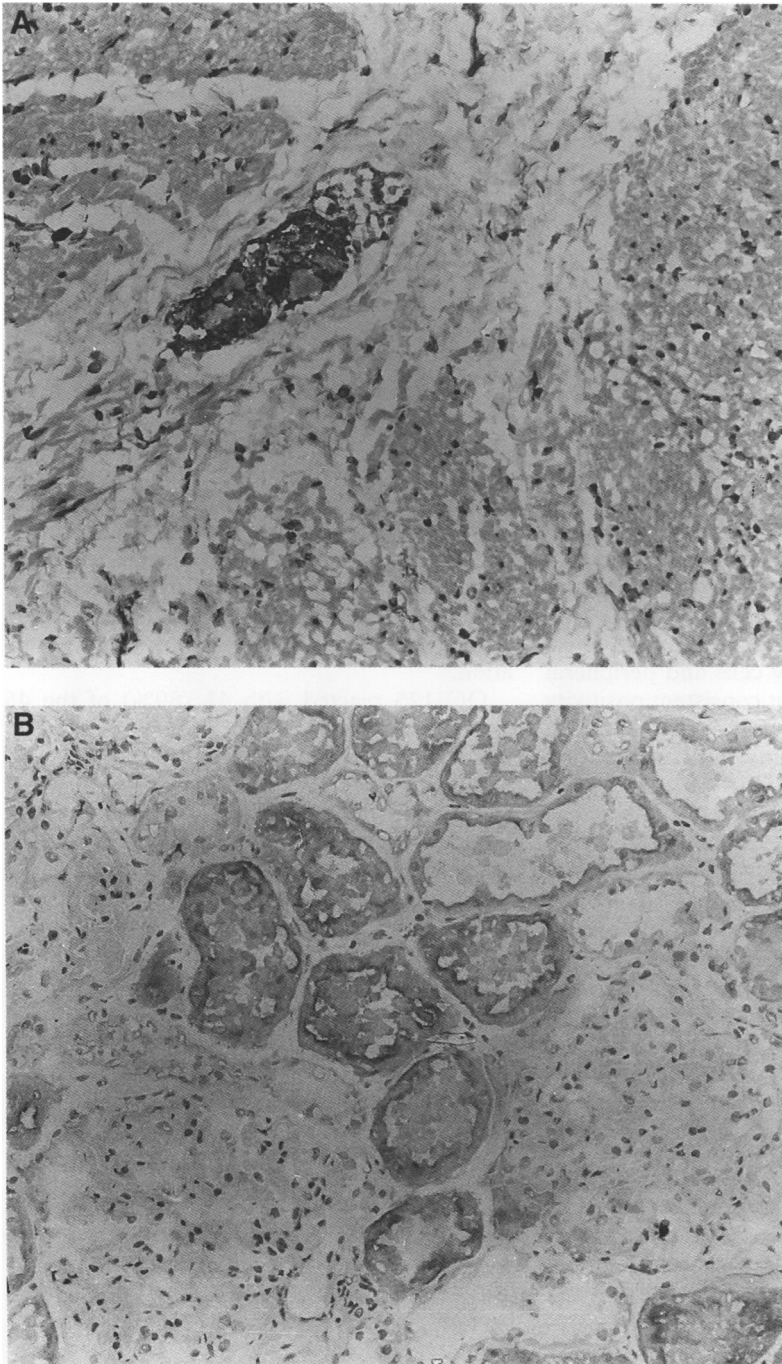


Figure 1 Immunostaining with OV-TL 3, showing (A) a positive reaction in the ganglion cells of the large intestine, and with MOv18 (B), showing a positive reaction in the distal tubuli of the kidney.

For immunofluorescence analysis,  $2.5 \times 10^5$  cells were incubated with 50  $\mu\text{g/ml}$  purified m- and c-MOv18 and 20  $\mu\text{g/ml}$  purified OV-TL 3, respectively, in ice-cold PBS/BSA for 45 minutes at  $0^\circ\text{C}$ . After washing in ice-cold PBS/BSA, cells were incubated with fluorescein isothiocyanate labelled goat antimouse immunoglobulin (GAM-Ig-FITC) (Becton Dickinson) for 30 minutes at  $0^\circ\text{C}$  in the dark. As controls for background fluorescence, cells were incubated with PBS alone and with GAM-Ig-FITC. After washing, cells were analysed immediately using FACScan (Becton Dickinson). Analysis was performed according to the manufacturer's instructions.<sup>18</sup> The results were described as fluorescence index (FI = (mean

fluorescence of the sample—mean fluorescence of the control)/mean fluorescence of the control).

To classify cells stained positively with OV-TL 3, a dual labelling procedure was performed on bone marrow cells and peripheral blood cells. OV-TL 3 was labelled with biotin according to the manufacturer's instructions (NHS-Biotin, Pierce Europe, Oud-Beijerland, The Netherlands). Glycerol (final concentration 50%) was added and the biotinylated monoclonal antibody was stored frozen at  $-20^\circ\text{C}$ . Dual labelling was performed by incubating cells with 20  $\mu\text{g/ml}$  biotinylated OV-TL 3, followed by an incubation step (45 minutes at  $0^\circ\text{C}$ ) with 25  $\mu\text{g/ml}$  FITC labelled anti-CD2, -CD3, -CD4, -CD8, -CD14, -CD16, -CD19, -CD34, and -CD38 (Becton Dickinson). Thereafter, cells were incubated for 15 minutes with 5  $\mu\text{g/ml}$  Streptavidin-phycoerythrin (PE). To correct for background fluorescence, the following control samples were included in the procedure: cells incubated with FITC labelled IgG, PE labelled IgG, FITC and PE labelled anti-CDs. Flow cytometry was performed as described earlier.

## Results

### NORMAL TISSUE DISTRIBUTION

Twenty eight different normal tissues were analysed for expression of OV-TL 3 and MOv18. The staining patterns found with OV-TL 3, c-MOv18, and m-MOv18 are shown in table 1. Multiple sections of each tissue type were tested and consistent results were obtained in all cases. No difference was observed in staining pattern between murine and chimeric MOv18.

With OV-TL 3, positive staining was observed in various tissue types (table 1). In contrast to the staining pattern of control IgG and MOv18, high background staining was observed in all tissues. A specific cell type or tissue responsible for this background staining could not be identified. In some cases the high background staining prevented reliable evaluation of the staining pattern. Staining with OV-TL 3 was mainly confined to cell membranes, but cytoplasm was stained faintly in almost all cases. OV-TL 3 reacted strongly (+ +) with the white cortex of the brain and moderately (+) with the perineurium of peripheral nerves. Moderate reactivity was also observed with the endothelial cells and the epithelium of sebaceous glands in the skin. The ciliated epithelium of the bronchi were moderately stained, whereas the pneumocytes were negative. In the intestine both the goblet cells and the plexus located in the submucosa of the intestine and between the longitudinal and inner circular muscle layers were stained positively (fig 1). Positive staining was also observed in the glomeruli of the kidney and transitional cells of the bladder. A positive reaction was also observed in epithelial cells of the normal ovary, the Fallopian tubes, the endometrium, and the cervix. Placental trophoblasts also stained positively. Weak reactivity was observed in some other tissues (table 1). On staining with OV-TL 3, a positive reaction

Table 2 FACSscan analysis following staining of peripheral blood, bone marrow, and spleen cells of healthy individuals with OV-TL 3, MOv18 and OC 125

Cell type	OV-TL 3		MOv18		OC 125	
	FI	Positive cells (%)	FI	Positive cells (%)	FI	Positive cells (%)
Peripheral blood lymphocytes						
Mean	19.7	95.5	0.2	1.9	0.6	3.4
SD	14.6	4.1	0.2	2.0	0.2	1.5
n	2	2	3	3	2	2
Bone marrow						
Mean	20.3	45.8	1.0	0.7	1.3	2.8
SD	12.6	5.6	1.1	1.4	0.9	4.0
n	4	4	4	4	4	4
Spleen						
Mean	10.5	51.3	0.6	0.6	2.8	7.7
n	1	1	1	1	1	1

was observed in lymph nodes, spleen, tonsil, and thymus. Further analysis was performed using flow cytometry. When OV-TL 3 was tested on fresh and fixed cell suspensions of peripheral blood, bone marrow and spleen, a clearly positive staining pattern was observed in leucocytes (table 2). Double staining with OV-TL 3 and the various immunological markers on bone marrow cells and peripheral blood showed variable but consistent positivity in both T and B cells (data not shown). Both the granulocyte and erythrocyte fractions were negative.

MOv18 reacted moderately (+) with the ciliated epithelium in the bronchi and weakly ( $\pm$ ) with the pneumocytes. The ductal epithelium and acinar cells of the pancreas stained positively. Furthermore, reactivity was observed with the distal tubuli in the kidney (fig 1). The follicular epithelium of the ovary, the epithelial lining of the Fallopian tubes, and the placental trophoblasts also stained positively. Weak reactivity ( $\pm$ ) was evident in some other tissues. In the thymus 10–20% of stromal cells stained weakly, whereas lymphocytes and Hassall bodies were negative (table 1). In all tissues staining with MOv18 was confined to the cell membrane. On flow cytometry, no immunoreactivity was observed with cells from peripheral blood, bone marrow or spleen.

#### OVARIAN CARCINOMAS

OV-TL 3 reacted positively with all 46 (100%) ovarian carcinomas tested. The staining intensity was strong in 33 and moderate in 11. Staining was very weak in two carcinomas. These tumours were derived from two patients who underwent primary debulking surgery with, respectively, moderately differentiated, mucinous, ovarian carcinoma and well differentiated, endometrioid carcinoma of the ovary. In most cases the carcinoma cells were homogeneously stained, with 90 to 100% of the cells being positive. There was no difference in staining pattern between different tumour deposits from the same patient, although in 12 patients the intensity of staining varied between different peritoneal metastases from faint to strong.

MOv18 reacted with 45 (98%) of the 46 ovarian carcinomas tested. The staining intensity was strong in 41 and moderate in three. Two moderately differentiated mucinous ovarian carcinomas showed very weak and neg-

ative staining, respectively. In positive tumours more than 75% of the tumour cells were stained. In four patients a difference in staining pattern, ranging from negative to moderately or strongly positive, between various peritoneal metastases was observed. In five patients the staining intensity varied from moderately to strongly positive depending on tumour location.

OC 125 reacted with 41 (89%) of the 46 ovarian carcinomas tested. Staining intensity was strong in 31, moderate in eight and weak in two. Tumours samples from five patients were completely negative, two of whom had recurrent disease diagnosed as poorly differentiated serous ovarian carcinomas, while the remaining three had primary disease diagnosed as well differentiated endometrioid, moderately differentiated mucinous and poorly differentiated serous ovarian carcinoma, respectively. The reaction pattern of an ovarian carcinoma tissue section on staining with OV-TL 3, MOv18 and OC 125 is shown in fig 2.

#### Discussion

In the present study normal and ovarian carcinoma tissues were analysed for their reactivity with OV-TL 3, m-MOv18 and c-MOv18. OV-TL 3 was previously reported by Poels *et al* to be non-reactive with skin, stomach, prostate, kidney, colon, liver, spleen, lung, and mesothelium.<sup>1</sup> Weak staining was found in the epithelial lining of the female genital tract such as the endocervix, endometrial glands, Fallopian tubes, and in some ovarian cysts. Our study confirms the reported reactivity in the female genital tract. We also observed reactivity in bronchial epithelium, endothelial and epithelial cells in the skin, goblet cells and ganglion cells in the intestine, ductal and acinar cells in salivary gland and pancreas, Langerhans' cells, Kupffer cells, glomeruli in kidney, transitional cells in the bladder, epithelial cells in breast, placenta, and prostate, brain cells, and peripheral nerves. The discrepancies might be explained by methodological differences. Poels *et al* used an indirect immunofluorescence assay, with possible fluctuations in specificity. Furthermore, background staining observed with OV-TL 3 might have obscured specific staining in normal tissues.

The gene encoding the OV-TL 3 defined antigen OA3 was cloned recently.<sup>10</sup> The antigen

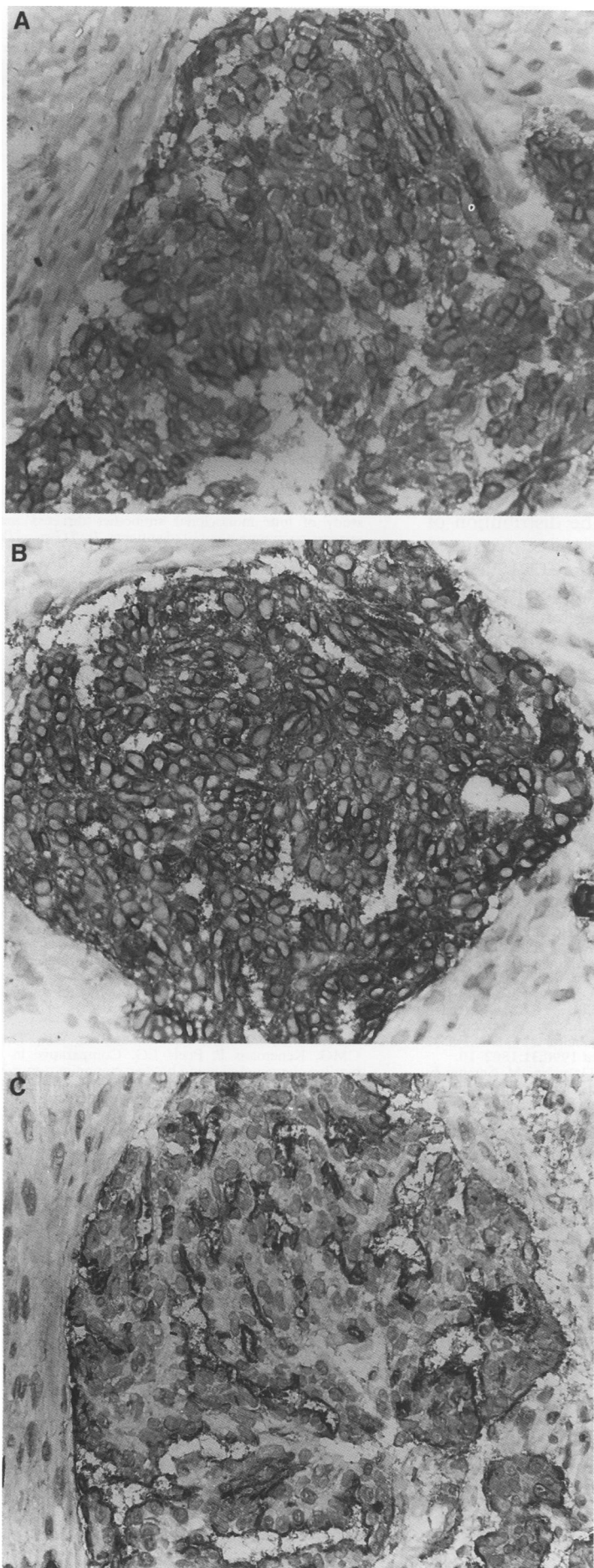


Figure 2 Reactivity of a poorly differentiated ovarian carcinoma tissue section with OV-TL 3 showing (A) a heterogeneous pattern and background staining, with MOv18 (B) showing homogeneous staining of the membrane, and with OC 125 (C) showing heterogeneous staining of the apical side of the tumour cells.

appeared to have a multimembrane spanning domain structure with an as yet unknown function, but which may have a role in membrane transport or signal transduction. On northern blot analysis, the OA3 messenger RNA (mRNA) was found in heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas.<sup>10</sup> In our immunohistochemical study heart and muscle tissue were negative on staining with OV-TL 3, but these differences could be due to the greater sensitivity of northern blot analysis.

Although m-MOv18 was originally reported to be restricted to ovarian carcinoma,<sup>2</sup> Stein *et al* showed immunohistochemical reactivity with ductal epithelium in the pancreas and the salivary glands, with Fallopian tube epithelium, cervical tissue, glandular cells in the bronchus, and with epithelial cells and/or secretions in the lung.<sup>13</sup> Our findings are in agreement with their data. The antigen recognised by MOv18, abundantly present on ovarian carcinoma cells, is a high affinity FBP.<sup>11,12</sup> Recently, Ross *et al* measured, using the polymerase chain reaction, the relative mRNA levels for the two known isoforms of FBP. Both forms were present in low to moderate quantities in a wide variety of normal and malignant tissues. More specifically, one of the isoforms was moderately expressed in normal colon, spleen, thymus and placenta, whereas the other isoform was highly expressed in ovarian carcinomas and some other tumours.<sup>19</sup> Our findings agree substantially with those of Weitman *et al*<sup>20</sup> who used immunohistochemical methods (MOv19) to stain FBP. In contrast to our findings with MOv18, these authors observed positive staining in the proximal tubules of the kidney, the acinar cells in the breast, and focal staining in the thyroid.<sup>20</sup> Mantovani *et al*<sup>21</sup> demonstrated, on staining with MOv18, intense and moderate staining in the Fallopian tubes and proximal and distal tubules, and weak or focal staining in the oesophagus, stomach, pancreas, mammary gland, and thyroid. Weitman *et al*<sup>22</sup> showed, using immunoblotting and radioimmunoassays, expression of FBP in the choroid plexus, thyroid, kidney, and lung. A possible correlation between MOv18 expression, the two FBP isoforms, and any clinical consequence is as yet unclear.

Radiolabelled OV-TL 3 and MOv18 have been used for radioimmunotargeting in patients with ovarian carcinoma.<sup>3,4,6,9</sup> The radiolabelled antibodies localised preferentially in ovarian cancer lesions, as shown by the positive immunoscintigrams and high uptake of radioactivity in tumour tissue compared with uptake in normal tissues, such as skin, muscle, fat, and normal peritoneum. Uptake in normal tissues observed on immunoscintigrams was as expected. No changes in haematological or chemical profiles were observed after injection of radiolabelled OV-TL 3 F(ab')<sub>2</sub> or c-MOv18.<sup>6,9</sup> OV-TL 3 was reported to be more useful for immunolocalisation compared with OC 125 because it is more homogeneously distributed within ovarian cancer tissue, the antigen is not shed, and is more favourably distributed in tumour bearing, athymic mice.<sup>23-26</sup>



In the present study OV-TL 3, MOv18 and CA 125 reacted with 100%, 98%, and 89% of the carcinomas examined, respectively. For each monoclonal antibody, the sensitivity was higher for serous ovarian carcinomas than with other types. Both findings agree with observations in other studies.<sup>15 16 27</sup> Heterogeneous staining intensity was observed within different peritoneal metastases of the same patient with all three monoclonal antibodies.

In antibody based immunotherapy, uptake of the monoclonal antibody in normal tissues might be detrimental when a high dose of antibody is required. However, it is unlikely that low level expression of an antigen in normal tissues will elicit side effects if the antibody directed against this antigen is applied clinically.<sup>28</sup> Nevertheless, with an immun-conjugate based on OV-TL 3, specific damage to leucocytes might cause serious side effects, limiting effective therapy. The distribution of MOv18 in normal tissue is more restricted than that of OV-TL 3, making MOv18 a more suitable candidate for immunotherapy. Therapeutic trials with this monoclonal antibody, however, will have to be carefully planned in view of the staining observed in the lung and kidney.

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- 1 Poels LG, Peters D, van Megen Y, Vooijs PG, Veihejen RHM, Willemsen A, *et al.* Monoclonal antibody against human ovarian tumor-associated antigens. *J Natl Cancer Inst* 1986;76:781-7.
- 2 Miotti S, Canevari S, Menard D, Mezzanzanica D, Porro G, Pupa SM, *et al.* Characterization of human ovarian carcinoma associated antigens defined by novel monoclonal antibodies with tumor restricted specificity. *Int J Cancer* 1987;39:1802-10.
- 3 Massuger LFAG, Kenemans P, Claessens RAMJ, Verheijen RHM, Schijf CPT, Strijk SP, *et al.* Immunoscintigraphy of ovarian cancer with indium-111 labeled OV-TL 3 (Fab')<sub>2</sub> monoclonal antibody. *J Nucl Med* 1990;31:1802-10.
- 4 Crippa F, Buraggi GL, Di Re E, Gasparini M, Seregni E, Canevari S, *et al.* Radioimmunoscintigraphy of ovarian cancer with the MOv18 monoclonal antibody. *Eur J Cancer* 1991;27:724-9.
- 5 Bolhuis RLH, Lamers CHJ, Goey SH, Eggermont AMM, Trimbos JB, Stoter G, *et al.* Adoptive immunotherapy of ovarian carcinoma with bs-MAb-targeted lymphocytes. A multicentre study. *Int J Cancer* 1992;48(Suppl 7):78-81.
- 6 Buist MR, Kenemans P, Vermorken JB, Golding RP, Burger CW, den Hollander W, *et al.* Radioimmunotargeting on ovarian carcinoma patients with indium-111 labeled monoclonal antibody OV-TL 3 F(ab')<sub>2</sub>: pharmacokinetics, tissue distribution, and tumor imaging. *Int J Gynecol Cancer* 1992;2:23-34.
- 7 Molthoff CFM, Buist MR, Kenemans P, Pinedo HM, Boven E. Experimental and clinical analysis of the characteristics of a chimeric monoclonal antibody, MOv18, reactive with an ovarian cancer-associated antigen. *J Nucl Med* 1992; 33:2000-5.
- 8 Coney LR, Mezzanzanica D, Sanborn D, Casalini P, Colnaghi MI, Zurawski VR. Chimeric murine-human antibodies directed against folate binding receptor are efficient mediators of ovarian carcinoma cell killing. *Cancer Res* 1994;54:2448-55.
- 9 Buist MR, Kenemans P, den Hollander W, Vermorken JB, Molthoff CFM, Burger CW, *et al.* Kinetics and tissue distribution of the radiolabeled chimeric monoclonal antibody MOv18 and (Fab')<sub>2</sub> fragments in ovarian carcinoma patients. *Cancer Res* 1993;53:5413-18.
- 10 Campbell IG, Freemont PS, Foulkes W, Trowsdale J. An ovarian tumor marker with homology to vaccinia virus contains an IgV-like region and multiple transmembrane domains. *Cancer Res* 1992;52:5416-20.
- 11 Campbell IG, Jones TA, Foulkes WD, Trowsdale J. Folate-binding protein in a marker for ovarian cancer. *Cancer Res* 1991;51:5329-38.
- 12 Coney LR, Tomassetti A, Carayannopoulos L, Frasca V, Kamen BA, Colnaghi MI, *et al.* Cloning of a tumor-associated antigen: MOv18 and MOv19 antibodies recognize a folate-binding protein. *Cancer Res* 1991;51: 6125-35.
- 13 Stein R, Goldenberg DM, Mattes MJ. Normal tissue reactivity of four anti-tumor monoclonal antibodies of clinical interest. *Int J Cancer* 1991;47:163-9.
- 14 Henzen-Logmans SC, Schipper NW, Poels LG, Kenemans P, Meyer CJLM. Use of statistical evaluation of antigen profiles in differential diagnosis between colonic and ovarian adenocarcinoma. *J Clin Pathol* 1988;41:644-9.
- 15 Berchuck A, Olt GJ, Soisson AP, Kanel A, Soper JT, Boyer CM, *et al.* Heterogeneity of antigen expression in advanced epithelial ovarian cancer. *Am J Obstet Gynecol* 1990;162: 883-8.
- 16 Boerman OC, van Niekerk CC, Makkink WK, Hanselaar AGJM, Kenemans P. Comparative immunohistochemical study of four monoclonal antibodies directed against ovarian carcinoma-associated antigens. *Int J Gynecol Pathol* 1991;10:15-25.
- 17 Bast RC, Klug TL, John E, Jenison E, Niloff JM, Lazarus H, *et al.* A radioimmunoassay using monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med* 1983;309:883-7.
- 18 van Dam PA, Watson JV, Lowe DG, Cox H, Curling M, Shepherd JH. Tissue preparation for simultaneous flow cytometric quantitation of tumour associated antigens and DNA in solid tumours. *J Clin Pathol* 1990;43:833-9.
- 19 Ross JF, Chaudhuri PK, Ratnam M. Differential regulation of folate receptor isoforms in normal and malignant tissues in vivo and in established cell lines. *Cancer* 1994;73: 2432-43.
- 20 Weitman SD, Weinberg AG, Coney LR, Zurawski VR, Jennings DS, Kamen BA. Cellular localization of the folate receptor—potential role in drug toxicity and folate homeostasis. *Cancer Res* 1992;52:6708-11.
- 21 Mantovani LT, Miotti S, Menard S, Canevari S, Raspagliesi F, Bottini C, *et al.* Folate binding protein distribution in normal tissues and biological fluids from ovarian carcinoma patients as detected by the monoclonal antibodies MOv18 and MOv19. *Eur J Cancer* 1994;30A:363-9.
- 22 Weitman SD, Lark RH, Coney LR, Fort DW, Frasca V, Zurawski VR, *et al.* Distribution of the folate receptor GP38 in normal and malignant cell lines and tissues. *Cancer Res* 1992;52:3396-401.
- 23 Haisma HJ, Knapp RC, Battaille A, Stradtman EW, Zurawski VR. Antibody-antigen complex formation following injection of I-131-labelled OC 125 monoclonal antibody F(ab')<sub>2</sub> fragments into patients with ovarian carcinoma. *Int J Cancer* 1987;40:758-62.
- 24 Boerman OC, Massuger LFAG, Makkink WK, Thomas CMG, Kenemans P, Poels LG. Comparative in vitro binding characteristics and biodistribution in tumor-bearing athymic mice of anti-ovarian carcinoma monoclonal antibodies. *Anticancer Res* 1990;10:1289-96.
- 25 Kenemans P. CA 125 and OA 3 as target antigens for immunodiagnosis and immunotherapy in ovarian cancer. *Eur J Obstet Gynecol Reprod Biol* 1990;36:221-38.
- 26 Molthoff CFM, Pinedo HM, Schluper HMM, Nijman HW, Boven E. Comparison of the pharmacokinetics, biodistribution and dosimetry of monoclonal antibodies OC 125, OV-TL 3, and 139H2 as IgG and F(ab')<sub>2</sub> fragments in experimental ovarian cancer. *Br J Cancer* 1992;65: 677-83.
- 27 Kuhnel R, Rao BR, Poels LG, Delemarre JFM, Kenemans P, Stolk JG. Multiple parameter analyses of human ovarian cancer: morphology, immunohistochemistry, steroid hormone receptors and aromatase. *Anticancer Res* 1988;8: 281-6.
- 28 Riethmuller G, Schneider-Gadicke E, Schlimok G, Schmiegel W, Raab R, Hoffken K, *et al.* Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. *Lancet* 1994;343: 1177-83.