ABSTRACT

A monoclonal antibody, designated NDOG2, has been shown to react with placental alkaline phosphatase. It does not bind to liver, kidney, bone, or intestinal alkaline phosphatase enzymes and has shown a positive reaction with all of over 50 placentas. Immunohistological studies have shown (a) a reaction with respiratory bronchiolar epithelium of lung, endocardial gland epithelium, the epithelium of fallopian tube, and certain reticular cells in the thymic medulla; (b) no detectable staining with ten other normal tissues including testis and tonsil; and (c) a significant reaction with nine of 13 ovarian cystadenocarcinomas and little or no staining with four malignant ovarian tumors of other histopathological types. The NDOG2 antibody may be of value as a marker in patients with ovarian cystadenocarcinoma.

INTRODUCTION

Placental alkaline phosphatase is a dimeric enzyme present in the placental syncytiotrophoblast plasma membrane from 10 to 16 weeks until term. Other human alkaline phosphatases also show restricted tissue distribution and have been grouped into a liver-kidney-bone type and an intestinal type. These different isoenzymes are conventionally distinguished by heat stability, inhibition with amino acids and peptides, and by the use of heterologous antisera (6). Peptide mapping studies have now provided definitive evidence for these 3 groups of isoenzymes and suggest that each is encoded by a separate genetic locus (18).

The placental enzyme is highly polymorphic. Six common forms are found in 98% of all placentae, deriving from 3 common alleles. The remaining 2% of placentae demonstrate rare variant enzymes. Over 40 such rare variants have so far been identified (5).

It was originally understood that the placental enzyme was not expressed in any other normal tissue (6). Recent reports, however, have demonstrated that extracts of lung, cervix, thymus, and testis contain trace amounts of a placenta-like alkaline phosphatase (4, 7). Similarly, placental enzyme is readily detectable in the sera of about 12% of all cancer patients and in some tumor extracts (17). A particularly high proportion of ovarian, testicular, breast, and cervical tumors gives rise to significantly elevated enzyme levels (3, 10, 12, 14, 17, 21). Some studies of tumor tissue have suggested a much higher enzyme incidence than is reflected by serum enzyme assays (2, 13, 21).

At least 50% of these tumor isoenzymes have properties distinguishable from the 6 common placental forms and may be analogous to the rare variants of the normal placenta and/or the forms present in lung, cervix, thymus, and testis (8). Conventional techniques are too imprecise to define accurately these tumor enzymes and relate them to placental or normal tissue analogues. This seriously limits the confidence with which this enzyme can be applied as a tumor marker. Furthermore, the published data chiefly concern enzyme detection in tumor extracts or patient sera. There are very few data on the localization of the enzyme at the histological level in either normal or tumor tissue. Such localization is essential for a correct interpretation of the enzyme expression in terms of cell differentiation and lineage.

MAbs2 recognize single molecular determinants. Antibodies to placental alkaline phosphatase thereby offer a rigorous control for specificity. Each MAb can be reproducibly defined in terms of its reaction with various isoenzymes, and so its clinical usefulness is ascertained. In addition, MAb s will allow precise definition of the number of antigenic determinants on the molecule and their expression on different enzyme variants. Considerable progress has already been made in this direction (16, 19). Also, MAbs provide rigorous specificity control between many different techniques, such as radioimaging, serum assay, and immunohistological localization.

We have developed a MAb to placental alkaline phosphatase and report here the detection and localization of enzyme in a series of normal and ovarian tumor tissues in an attempt to assess the clinical potential of this antibody as a marker for ovarian cancer.

MATERIALS AND METHODS

Immunohistology. Adult tissues were obtained from postmortem material within 24 hr of death or fresh from surgery (see Table 2). Placentae were obtained fresh either after suction aspiration or pregnancy hysterectomy, for placentae of 8 to 16 weeks menstrual age, or after vaginal delivery or cesarean section from 24 weeks until term. Small pieces, approximately 0.5 mm in diameter, were frozen by placing them on aluminum foil and floating the foil on liquid nitrogen. They were then placed in cryotubes under liquid nitrogen or at −70°C until use. Frozen sections 5 to 8 μm were cut, air dried, for 0.5 to 2 hr, fixed for 10 min in acetone at room temperature, and washed in PBS for 2 min. Fifty μl of MAb at a predetermined, saturating concentration were then applied to each section, incubated 45 min, washed twice in PBS (2 min), and incubated with 50 μl of peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako) in PBS/10% normal human serum (1/50) for 30 min. Sections were washed again and incubated with 50 μl of diaminobenzidine tetrahydrochloride (Sigma) in PBS (0.5 mg/ml)/0.007% H2O2 for 8 min. They were then washed in excess water, counterstained with Mayer’s hematoxylin, and mounted in DPX (BDH Chemicals). In addition, one set of sections was subjected to conventional hematoxylin/eosin staining.

In all cases, specific NDOG2 staining was assessed relative to a panel

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2 The abbreviations used are: MAb, monoclonal antibody; TBS, Tris-buffered saline (0.15 M NaCl/0.05 M Tris-HCl, pH 7.6).
Table 1

<table>
<thead>
<tr>
<th>MAb</th>
<th>Immunoglobulin type</th>
<th>Specificity</th>
<th>Antigen distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDOG2</td>
<td>Mouse IgG2b</td>
<td>Placental alkaline phosphatase</td>
<td>Tissue</td>
</tr>
<tr>
<td>W6/32</td>
<td>Mouse IgG2</td>
<td>Human Class I HLA antigens</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td>TROMA1</td>
<td>Rat IgG</td>
<td>Intermediate filament protein</td>
<td>Differentiated structures including many epithelia</td>
</tr>
<tr>
<td>BMH1</td>
<td>Mouse IgM</td>
<td>Unknown</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td>MRC OX6</td>
<td>Mouse IgG</td>
<td>Rat Class II histocompatibility antigens</td>
<td>Absent from all human tissues</td>
</tr>
<tr>
<td>NDOG4</td>
<td>Mouse IgG1</td>
<td>Unknown</td>
<td>Trophoblast and a small no. of uncharacterized cells in some adult tissues</td>
</tr>
</tbody>
</table>

Placental Alkaline Phosphatase and Ovarian Cancer

The Table 1 shows the monoclonal antibodies used in the study. The antibodies include NDOG2, W6/32, TROMA1, BMH1, MRC OX6, and NDOG4. The specificities of these antibodies range from placental alkaline phosphatase to human class I HLA antigens and intermediate filament proteins.

Antibody Specificity

Charts 1 and 2 illustrate the specificity of the antibodies. Chart 1 shows the results of a microtiter plate assay, indicating that NDOG2 specifically binds to placental alkaline phosphatase. Chart 2 demonstrates the inhibition of this binding by the addition of normal human serum.

RESULTS

Placenta. The study also examined placental alkaline phosphatase activity, which was specifically bound to NDOG2 from several normal sera tested, whether or not they were preheated.

Immunohistology of Normal Tissues

In the placenta, NDOG2 binds to the syncytiotrophoblast membrane of the human placenta in the last trimester of pregnancy, but not to placentae of 8 weeks menstrual age. Washing was then followed by development of a colorimetric reaction for alkaline phosphatase using p-nitrophenyl phosphate as substrate.
villous syncytiotrophoblast plasma membrane (results not shown). A lesser, variable amount of staining was also occasionally found on the syncytiotrophoblast basement membrane in the second and third trimesters. Nonvillous forms of trophoblast in the placental bed bound either no or very low quantities of NDOG2 as detected by immunoperoxidase staining. In the chorionic membrane at term, up to 50% of cytotrophoblast cells bound NDOG2, and these cells were concentrated on the fetal aspect of the chorionic trophoblast layer (not shown). NDOG2 has been found to react with all of more than 50 placenta taken fresh from surgery and from postmortem tissue, respectively.

Other Normal Tissues. A small number of human tissues have been examined with the NDOG2 MAb. The results are presented in Table 2. Most tissues were found not to bind detectable amounts of NDOG2 antibody. Specific staining was, however, detected in lung, cervix, thymus, and fallopian tube. Examples of this are given in Fig. 1. In lung tissue, NDOG2 reactivity varied markedly among 6 specimens examined. In 3, there was no reaction, including one sample containing bronchus and associated alveoli. In 3 others, clear, specific staining was apparent, and in one case, this was localized to respiratory bronchiolar epithelium proceeding from a terminal bronchiole (Fig. 1a). The specific staining was found not to circumscribe the epithelial cells as would be expected for a typical cell membrane antigen but, rather, presented as a linear streak on a single epithelial surface. Such a staining pattern may be consistent with enzyme secretion. In the cervix, there was some nonspecific staining of endothelial gland secretions. This was reduced by incorporation of normal human serum in the MAb incubation so as to reveal specific NDOG2 binding to the luminal epithelial surface (Fig. 1, b and c). Similarly, the reactivity of the fallopian tube was confined to the luminal epithelial surface (Fig. 1d). Thymus gave the only example of a normal cellular reactivity. A minor subpopulation of reticular cells in the thymic medulla gave a positive reaction which was diffuse throughout the cell, probably indicating cytoplasmic localization of antigen. These cells comprised <1% of the cells in the thymus and were of typical reticular morphology, having a large pale-staining nucleus and long cytoplasmic processes (Fig. 1e).

Ovarian Tumors. A series of 21 malignant tumors has been examined for reactivity with the NDOG2 MAb. The results are summarized in Table 2. Nine of 13 ovarian cystadenocarcinomas gave significant NDOG2 staining as defined by reactivity of more than 1% of neoplastic epithelial cells (Table 3, Grade ++ or more). An additional 2 of 4 adenocarcinomas of uncertain, but possibly ovarian, origin were positive.

The NDOG2 antibody bound exclusively to malignant epithelial cells in all the tumors examined, leaving the fibrous stromal tissue unstained (Fig. 2, a and b). The epithelial cells of serous cystadenocarcinomas demonstrated a typical cell membrane reactivity (Fig. 2a). In addition, one mucin-secreting papillary cystadenocarcinoma also demonstrated some cytoplasmic staining as well as cystic areas containing considerable NDOG2-reactive material in the lumen (Fig. 2b). This was accompanied by a varying degree of cystic epithelial degeneration.

The other histopathological types of malignant tumor examined showed only very little or no NDOG2 reactivity. In a mesonephroid tumor, 2 distinct areas were observed. One of these was a solid mass of NDOG2-negative tumor cells, the other was cystic, and a varying degree of specific NDOG2 staining was observed on the epithelial cells lining the cysts (Fig. 2c). Two tumors of borderline cancer have been studied to date. One, designated serous papillary cystadenoma, showed considerable epithelial proliferation with no evidence of stromal invasion. These proliferating epithelial cells showed foci of intense NDOG2 reactivity illustrated in Fig. 2d. In contrast, a tumor designated early invasive mucinous cystadenocarcinoma by conventional histopathology was negative for NDOG2, although only small areas of epithelium were examined by immunohistochemistry, and none of these demonstrated either atypical proliferation or stromal invasion.

Histochemistry. Serial sections of the serous papillary cystadeno- enoma, illustrated in Fig. 2d, were stained by the immunoperoxidase technique with NDOG2 and histochemically for the en- zyme alkaline phosphatase. The alkaline phosphatase reaction was intensely positive on precisely the same subpopulation of epithelial cells as bound detectable NDOG2 antibody. This en- zymatic activity was totally inhibited by I-phenylalanylglycylglycine (2 mm), while levamisole (1 mm) gave only a marginal diminution in staining. These inhibitory properties are characteristic of pla- cental-type alkaline phosphatase as determined by parallel ex- periments on term placental sections and from the reports of other workers (7).

Stromal tumor tissue showed a lesser amount of alkaline
Phosphatase activity localized on blood vessel endothelium which was completely inhibited by levamisole (1 mm) but unaffected by L-phenylalaninoglycylglycine (2 mm). This is typical of the liver-kidney-bone type of the alkaline phosphatase isoenzyme. The NDOG2 antibody did not bind to any stromal tissue.

Similar studies were performed on duodenum, liver, and kidney. Alkaline phosphatase activity was clearly demonstrable on the intestinal epithelium, the sinusoids of liver, and kidney tubules. Serial sections of these tissues gave no reaction with NDOG2 in the indirect immunoperoxidase technique, despite morphological integrity of the enzymically active cells and their positive staining by TROMA1 MAb (duodenum) or W6/32 (kidney sinusoids) and liver sinusoids). NDOG2 therefore does not cross-react with the intestinal or liver-kidney-bone forms of alkaline phosphatase.

**DISCUSSION**

Chart 1 demonstrates that NDOG2 binds to a determinant of placental alkaline phosphatase. One previous report of the absence of NDOG2 reactivity in pregnant serum was due to insufficient sensitivity in the assay system used. The appearance and distribution of NDOG2 reactivity on placenta are entirely in accord with the established properties of placental alkaline phosphatase, and the fact that NDOG2 reacted with all of more than 50 placenta studies implies that the recognized determinant is shared among the 3 common polymorphic variants of the enzyme monomer. The histochemical studies demonstrate that NDOG2 does not cross-react with the liver-kidney-bone or intestinal forms of alkaline phosphatase.

We have been able to localize the enzyme in normal lung, cervix, fallopian tube, and thymus. In each case, enzyme is associated with only a minor cell population in the tissue, and in lung, cervix, and fallopian tube, the staining pattern may be indicative of its presence in epithelial secretions. This concurs with previous work showing the existence of trace quantities of placenta-like alkaline phosphatase in extracts of lung, cervix, thymus, and testis (7). To our knowledge, no one has studied extracts of fallopian tube for enzyme activity. We have not yet detected NDOG2 staining in testis. This may be because NDOG2 does not react with the enzyme variant present in testis, although it is noteworthy that one specimen of seminoma thus far studied gave intense, generalized NDOG2 staining (Table 3, equivalent to Grade ++++). Alternatively, enzyme might be present on some testis-associated structure which we have not yet studied, such as the appendix testis, rete testis, or epididymis. One other study has reported placental alkaline phosphatase on mitogen- or alloantigen-activated lymphocytes (9). Our results do not bear directly on this question, although we note that on nontoxic lymphocytes reacted with NDOG2 in tissue sections, and previous studies have demonstrated no significant in vitro binding of NDOG2 antibody to the whole population of unstimulated peripheral blood lymphocytes (20).

It is apparent that NDOG2 MAb binds to a substantial proportion of ovarian cystadenocarcinomas. These account for up to 70% of ovarian cancers, and NDOG2 may therefore be of some clinical value in ovarian cancer. It is likely that, as suggested by other studies (2, 13, 21), examination of tumor tissue itself may be a more sensitive index of marker expression than serum assay. The immunohistochemical technique may therefore be of value in selecting out a population of patients on the basis of marker detection on the primary tumor. This population might then be usefully followed up by serum assay and radioimaging using the MAb in an attempt to monitor the onset and progress of secondary disease. Preliminary studies of radioimaging using 125I-labeled NDOG2 antibody have shown uptake of antibody to known sites of secondary ovarian cancer and no specific uptake by any normal tissue including lung (22).

Throughout this work, we have used fresh, frozen, tissue sections. Attempts to use conventionally processed formalin-fixed, wax-embedded material have given variable results consistent with partial denaturation of the NDOG2 antigenic determinant. Future studies with this antibody will therefore require fresh, frozen tissue.

Finally, it is of interest to consider the developmental lineage of the cells producing placental alkaline phosphatase. In the female, the cells of the paramesonephric ccelomic epithelium go to form the müllerian duct which, in turn, gives rise to the various epithelial linings of the endocervix, endometrium, fallopian tube, and the ovary. Both the fallopian tube and endocervix epithelia normally express placental alkaline phosphatase, while those of the cervix and ovary frequently express enzyme after malignant transformation. It is tempting to speculate that cells derived from the paramesonephric ccelomic epithelium may have a particular tendency towards placental alkaline phosphatase expression. While this idea is clearly limited in that it does not explain enzyme expression by other tumors or normal tissues, it may serve as an argument against the hypothesis that feto/placental antigens are reexpressed by tumors as a result of a totally random depression of genes.

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**REFERENCES**

11. Kemler, R., Brulet, P., and Jacob, F. Monoclonal antibodies as a tool for the


Fig. 1. Frozen sections of normal human tissues were stained with MAbs by an indirect immunoperoxidase technique. Nuclei were counterstained with hematoxylin. a, NDOG2 on lung tissue demonstrating reaction with one surface of respiratory bronchiolar epithelium proceeding from an unstained terminal bronchiole (TB); b, NDOG2 on the endocervix showing staining of the luminal surface of epithelium; c, serial section of endocervix stained with MRC OX8, as a negative control; d, NDOG2 on fallopian tube demonstrating reaction with the luminal epithelial surface; e, NDOG2 on thymus showing staining of some reticular cells close to a Hassal's corpuscle (HC) in the thymic medulla. d and e were photographed through phase contrast. Scale bar, 50 μm (a to c) or 10 μm (d and e).
Fig. 2. Frozen sections of ovarian tumor tissue were stained with the NDOG2 MAb by an indirect immunoperoxidase technique. Nuclei were counterstained with hematoxylin. a, well-differentiated serous papillary cystadenocarcinoma. The majority of neoplastic epithelial cells are stained. There is no staining of the surrounding stromal tissue. b, mucin-secreting papillary cystadenocarcinoma showing staining on the luminal surface of glandular epithelium. c, mesonephroid tumor in which the cystic epithelium showed a varying but low degree of specific reactivity. d, serous papillary cystadenoma showing considerable epithelial proliferation. A subpopulation of epithelial cells demonstrates intense NDOG2 reactivity. Scale bar, 50 μm.
Immunohistology of Normal and Ovarian Cancer Tissue with a Monoclonal Antibody to Placental Alkaline Phosphatase

Christopher A. Sunderland, John O. Davies and Gordon M. Stirrat


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