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Antibodies to the 280-kd Coated Pit Protein, Target of Teratogenic Antibodies, Produce Alterations in the Traffic of Internalized Proteins

S. Le Panse,* E. Ayani,† N. Mulliez,†
F. Chatelet,‡ C. Cywiner-Golenzer,‡
M. Galceran,* D. Citadelle,† Ch. Roux,†
P. Ronco,* and P. Verroust*

INSERM U 64 Hôpital Tenon; Département de foetopathologie,† Hôpital Saint Antoine; and Laboratoire d'anatomie-pathologique,‡ Hôpital Rothschild, Paris, France*

Previous studies have identified two high-molecular weight (280 and 330 kd) glycoproteins expressed by coated pits of the proximal renal tubule and yolk sac and have further established that, in vivo, antibodies to gp280 but not to gp330 induce fetal malformations. In the present study, we report the effect of these antibodies on the endocytic process by yolk sac visceral epithelial cells of rat embryos explanted at day 10 of gestation. Antibodies to gp280 markedly altered development of the yolk sac and embryo, induced malformations, inhibited by 40% the uptake of [^{14}C] sucrose and perturbed the intracellular traffic of internalized proteins. Under control conditions, rat immunoglobulin G present in the culture medium was immunolocalized in lysosomes of epithelial cells, whereas in the presence of antibody, it was detected in small vesicles scattered through the apical cytoplasm. Alterations of the endocytic pathway were confirmed by experiments analyzing the uptake of peroxidase added to the medium for 2 to 60 minutes. The initial compartments of endocytosis visualized by peroxidase were increased in size and abnormal in shape and the transfer of the internalized peroxidase to the lysosomal compartment was delayed. In contrast, antibodies to gp330 had a minimal effect on embryonic development and did not induce fetal malformations. Endocytosis was only modestly altered; uptake of [^{14}C] sucrose was decreased by 25%, and only minor modifications of the intracellular transit of peroxidase could be detected. We suggest that the key role of anti-gp280 antibodies is via trapping of the target antigen in the early endocytic compartment thus preventing its normal function in lysosomal transfer. (Am J Pathol 1994, 145:1526–1536)

More than 30 years ago, Brent et al[1] and David et al[2] reported that antibodies raised against the kidney induced fetal malformations when injected during the early period of organogenesis. These observations have been confirmed and extended by a number of researchers and laboratories. Efforts were first directed toward an understanding of the mode of action of the antibodies and identified the visceral yolk sac as the target. This organ plays a key role in fetomaternal exchanges, since it is the only interface exhibiting a placental function during the first 10 days of pregnancy.[3] It is endowed with high endocytic properties and ability to degrade internalized proteins, and studies by Lloyd and coworkers[4–9] have shown that the only significant source of amino acids used by the 10th-day embryo is protein taken up by the yolk sac and digested intracellularly. The observation, using *in vitro* systems, that polyclonal teratogenic antibodies[10] decreased internalization of fluid phase markers, led to the conclusion that they acted by decreasing the supply of nutriments to the embryo via diminished uptake of protein. The significance of these observations was underscored by the fact that among monoclonal anti-yolk sac antibodies,[11] only those that induced decreased endocytosis were teratogenic.

In addition to these experiments, various groups searched for specific protein(s) that might be the target(s) of the teratogenic antibodies. Leung[12] identified a 320-kd protein from renal brush border, which was initially defined as diffusely expressed by the

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Address reprint requests to P. J. Verroust, INSERM U 64 Hôpital Tenon, 4 Rue de la Chine 75020 Paris, France.

brush border and subsequently[13] as restricted to the clathrin-coated intermicrovillar areas. Our group[14] reported that antibodies to a 280-kd protein concentrated[15] in the clathrin-coated intermicrovillar area of the proximal tubule and yolk sac induced fetal malformations whereas, even at high dosages, antibodies to a 330-kd protein[16,17] initially identified as the antigen responsible for Heymann's nephritis and characterized by a similar subcellular distribution failed to do so. Jensen et al[11] reported several teratogenic monoclonal antibodies but could not define the target antigen. Although the proteins described by Leung and our group may be identical,[18] their reactivity with the antibodies described by Jensen et al[11,19] is unknown and it has been proposed that several antigens might serve as targets.

In the present study we have analyzed *in vitro* the effect of monoclonal anti-gp280 and anti-gp330 antibodies on the endocytic process. We report that anti-gp280 antibodies decreased the amount of [^{14}C] sucrose internalized but also induced striking modifications of the endocytic compartments visualized by peroxidase. Antibodies to gp330 also influenced the endocytic process but to a considerably lesser extent. They had little effect on embryonic growth and did not induce fetal malformations.

Materials and Methods

Embryo Cultures

Cultures were carried out as previously described by New et al[20] with minor modifications. Wistar rat embryos were dissected free of the uterine wall on the 11th day of gestation and placed after removal of the parietal layer of the yolk sac in round flasks containing 2 ml of heat-inactivated rat serum under constant rotation for 48 hours, during which the concentration of O_2 was progressively increased.

Antibodies

The monoclonal antibodies used in this study have been previously reported.[14,17,21] MAb 75 is specific for gp280 and MAb 12 for gp330. MOPC21, a monoclonal immunoglobulin G (IgG) devoid of known specificity, was used as a control.

In Vitro *Teratogenic Effect of Antibodies*

In a first series of experiments, we tested the effects of antibodies on embryonic development. For this purpose, simultaneous cultures were carried out under control conditions (rat serum only) or in the presence of rat serum supplemented from the beginning of the experiment with the monoclonal antibody tested at concentrations of 20 to 200 μg/ml.

At the end of the culture, the embryos were carefully dissected under a stereo microscope to appreciate growth and differentiation. The diameter of the yolk sac, length of the head, head-to-tail distance, number of somites, and differentiation score of Brown and Fabro[22] were recorded.

Ultrastructural Morphology and Localization of Teratogenic Antibodies

To assess ultrastructural morphology, yolk sacs were fixed at the end of the culture with 0.5% glutaraldehyde in cacodylate buffer for 1 hour at 4 C, processed, and examined with a Zeiss (Carl Zeiss S. A. 78 Le Pecq, France electron microscope as previously described.[23] In addition, experiments were carried out to localize cell-bound teratogenic mouse IgG added in the culture medium. For this purpose, at the end of the culture period, yolk sacs were fixed in periodate-lysine-paraformaldehyde containing 4% paraformaldehyde[24] and incubated sequentially with biotin-labeled species-specific anti-mouse IgG followed by peroxidase-labeled streptavidin (Amersham-France, les Ulio, France). Detection of bound peroxidase by diaminobenzidine and subsequent tissue processing were as previously reported.[23] Acid phosphatases were determined according to Boutry and Novikoff[25] on tissues fixed for 2 hours at 4 C with 1% glutaraldehyde.

Internalization of [^{14}C] Sucrose

Internalization of [^{14}C] Sucrose was measured during the final 6 hours of the 48-hour cultures. For this purpose, 1 μCi of [^{14}C] sucrose was added to the culture medium after 42 hours of culture. After a further incubation of 6 hours, embryos were removed from the medium and washed in Hanks' balanced salt solution (HBSS). Yolk sacs were carefully dissected and dissolved in 1 N NaOH. A sample was used for protein determination according to Lowry,[26] and ^{14}C radioactivity was determined on the remaining material. A sample of the medium was also saved for counting.

Results were expressed as previously reported,[4,8] as μl fluid internalized per hour and per mg protein. Experiments were performed on cultures that had been exposed to antibody to gp280 (60 μg/ml) from

the first hour or in cultures in which antibody to gp280 or gp330 was added during the final 6 hours of culture.

Internalization of Rat IgG

Experiments were carried out to localize in the cells the rat IgG contained in the culture medium. For this purpose, yolk sacs were processed as described above for the detection of teratogenic mouse antibodies except for the use of biotin-labeled species-specific anti-rat IgG (Amersham).

Internalization of Peroxidase

To visualize sequentially the intracellular compartments containing internalized material, cultures were incubated in the presence of peroxidase (Sigma Chemical Co., St. Louis, MO) for various periods of time ranging from 2 to 60 minutes. At the end of the incubation period, embryos were washed rapidly in cold HBSS and transferred to cacodylate buffer containing 0.5% glutaraldehyde. Yolk sacs were carefully dissected and 1) kept in the same fixative for 1 hour at 4 C, 2) transferred to cacodylate buffer and incubated with diaminobenzidine to reveal peroxidase, and 3) treated with reduced osmium and embedded in Epon using routine techniques in the laboratory.[23] Experiments were performed on yolk sacs that had been exposed to antibody to gp280 or gp330 (60 μg/ml) during the entire culture period and, in the case of antibody to gp280, only during the final 6 hours of culture. A minimum of four experiments (including at least one yolk sac) were carried out for each time point and each experimental condition.

Quantitative Morphometry

Quantitative morphometric analysis of peroxidase-containing vesicles was carried out on 0.35-μ semithin sections cut with a diamond knife. Slides were examined under bright field conditions using a 100× objective to select fields containing transversal sections of whole cells, most often three or four per field. For each field studied, the contours of the cells analyzed were manually defined, and artifacts due to resin embedding were identified and eliminated. The density level of peroxidase reaction product was defined once for all the experiments. A minimum of three fields were studied for each yolk sac, and the results were expressed as the mean ratio of peroxidase-containing vesicles per total cell area.

Results

In Vitro Teratogenic Effects of Anti-GP280 Antibodies

In a first series of experiments, we tested the *in vitro* effect on embryonic development of antibodies to coated pit glycoproteins. The overall results are presented in Table 1. As can be seen, even at the lowest concentration used, the presence of MAb75, specific for gp280, inhibited the development of the yolk sac and of the embryo. In addition, detailed study of the embryos revealed malformations in all the 40 embryos studied, involving essentially the eye and cephalic pole. Doses higher than 120 μg/ml had a lethal effect on the embryo. Control Ig such as MOPC21 had no effect on the development of the embryo. Antibodies (MAb12) specific for another coated pit protein, gp330, only had a mild effect on embryonic development and did not induce fetal malformations.

Table 1. *Development of Embryos Cultured in vitro in the Presence of Monoclonal Antibodies to gp330, gp280, or Control Mouse IgG*

Antibody	Number of Experiments	Score*	Yolk Sac Diameter (mm)	Head-to-Tail Distance (mm)	Head Length	Somites	Fetal† Malformations
Control	74	49.7 ± 3.9	5.8 ± 0.5	5.0 ± 0.5	2.8 ± 0.3	34.1 ± 2.8	0
MOPC	14	46.0 ± 2.0	6.1 ± 0.7	4.9 ± 0.6	2.8 ± 0.5	35.3 ± 2.1	0
MAb 12							0
100 μg/ml	9	46.3 ± 9.4	5.8 ± 0.6	4.8 ± 0.8	2.8 ± 0.4	34.1 ± 1.1	
200 μg/ml	3	31.3 ± 7.0	5.2 ± 0.6	3.7 ± 0.4	2.1 ± 0.3	ND	
MAb 75							
20 μg/ml	9	45.4 ± 5‡	5.1 ± 0.4§	4.6‖ ± 0.4	2.5 ± 0.4‡	ND	40/40
60 μg/ml	31	39.3 ± 0.6§	4.7 ± 0.4§	4.0 ± 0.5§	2.1 ± 0.4§	28.9 ± 4.3§	

* The development of the following elements is graded on a scale from 1 to 4: yolk sac vascular system; allantois; flexion of the embryo; heart; anterior, middle, and posterior brain; axial and caudal nervous systems; optical system; olfactive apparatus; branchial arch's; mandibular and maxillar processes; and anterior and posterior limbs. The score in this table is the sum of these 16 individual scores as described by Brown and Fabro.[22]

† Anophtalmia, microphtalmia, telecephalic hypotrophy, cephalic-neural tube defects, hydrops.

‡ $P < 0.003$; § $P < 0.001$; ‖ $P < 0.05$

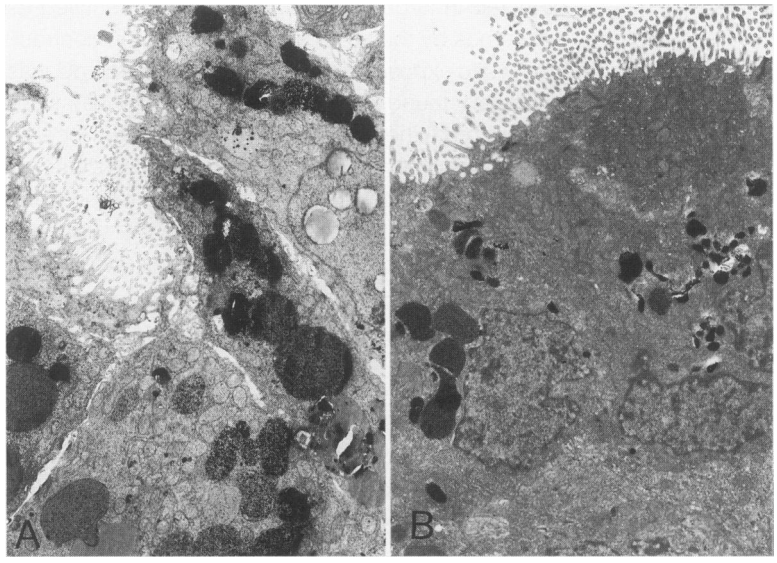


Figure 1. *Immunohistochemical localization of acid phosphatases in epithelial cells of yolk sac cultured under control conditions* (**A**) *or in the presence of antibody to gp280* (**B**). *Note that cells in* (**B**) *have a normal morphology but contain lysosomes of smaller size and of abnormal shape. Original magnifications: ×3000.*

Morphology of Yolk Sacs

Epithelial cells of yolk sacs cultured under control conditions were characterized by a tall and well-developed brush border, small and large endocytic vacuoles, and, as shown in Figure 1A, a large number of vesicles containing acid phosphatases that could thus be classified as lysosomes. Epithelial cells from yolk sacs cultured in the presence of antibodies to gp280 for 48 hours had a normal morphology but contained a smaller number of large vesicles staining for acid phosphatases (Figure 1B). In addition numerous vesicles of abnormally small size and irregular shape were stained for acid phosphatases.

Cell morphology, including number, size, and shape of lysosomes, was normal in cultures carried out in the presence of antibodies to gp330 or after short (6-hour) incubations in the presence of antibody to gp280 (data not shown).

Immunolocalization of MAb75

As shown in Figure 2, when yolk sacs were cultured in the presence of antibody to gp280, the mouse IgG was detected in the intermicrovillar areas, in large and small endocytic vesicles and in lysosomes. When cultures exposed to MOPC 21 were examined, reaction product was only detected in lysosomes (data not shown).

Internalization of [^{14}C] Sucrose

The amount of sucrose internalized by yolk sacs was measured over a period of 6 hours on embryos cultured under control conditions or in the presence of antibodies to gp280 or gp330. As shown in Table 2 the amount of fluid internalized was decreased by 40% when cultures had been exposed to antibody to gp280 for 48 hours. Since, under these conditions, yolk sacs were atrophic and contained abnormal lysosomes as indicated by ultrastructural studies, measurements of [^{14}C] sucrose uptake were repeated on cultures exposed to antibody only during the last 6 hours of culture (ie, antibody was added at

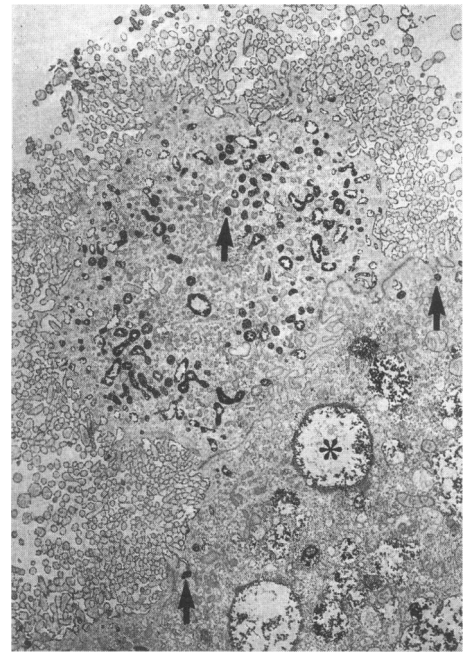


Figure 2. *Immunolocalization of MAb75 in epithelial cells of yolk sac cultured in the presence of antibodies to gp280 for 48 hours. On this tangential section note localization of mouse IgG in coated pits/membrane invaginations* (*arrows*) *and in large endocytic vacuoles* (•). *Original magnification: ×3000.*

Table 2. *Effect of Anti-yolk Sac Antibodies on the Pinocytic Uptake of ^{14}C Sucrose by Day 12 Rat Visceral Yolk Sacs*

Antibody	Exposure (hours)	No. Experiments	Endocytic Index*
Control		23	3.97 ± 2.0
MAb 75	48	10	1.86 ± 0.5†
MAb 75	6	18	1.86 ± 1.37†
MAb12	6	20	2.52 ± 0.85‡

* µl/mg of visceral yolk sac protein.
† $P < 0.001$.
‡ $P < 0.02$.

the same time as sucrose). The amount of sucrose internalized (Table 2) was again significantly lower than in control cultures. When similar experiments were carried out with antibodies to gp330, uptake of [^{14}C] sucrose was also significantly decreased but to a lesser extent.

Internalization of Tracers

Rat IgG

Initial experiments were carried out using as tracer the rat IgG contained in the culture medium. As shown in Figure 3A, under control conditions rat IgG was detected in large vesicles, which were identified as lysosomes by their size and localization. In contrast, in yolk sac exposed to antibody for 48 hours, IgG was essentially found in small vesicles (Figure 3B), scattered through the apical cytoplasm, but often located close to the brush border. Rat IgG was also detected in large endocytic vacuoles and occasionally in a network of tube-like structures located in the subapical area (Figure 3C). Only rare lysosome-like structures were found.

Peroxidase

To study the initial steps of endocytosis, further experiments were carried out on yolk sacs cultured in the presence of antibody to gp280 for 48 hours and exposed to peroxidase for 2 to 60 minutes. As shown in Figure 4, peroxidase was very rapidly internalized by yolk sac epithelial cells in both control and antibody-exposed cultures, but the intracellular vesicular structures in which it was contained were morphologically distinct. In control cultures, as early as 2 minutes after addition of the tracer to the medium, peroxidase could be detected (Figure 4A) in small polymorphic endocytic vesicles under the brush bor-

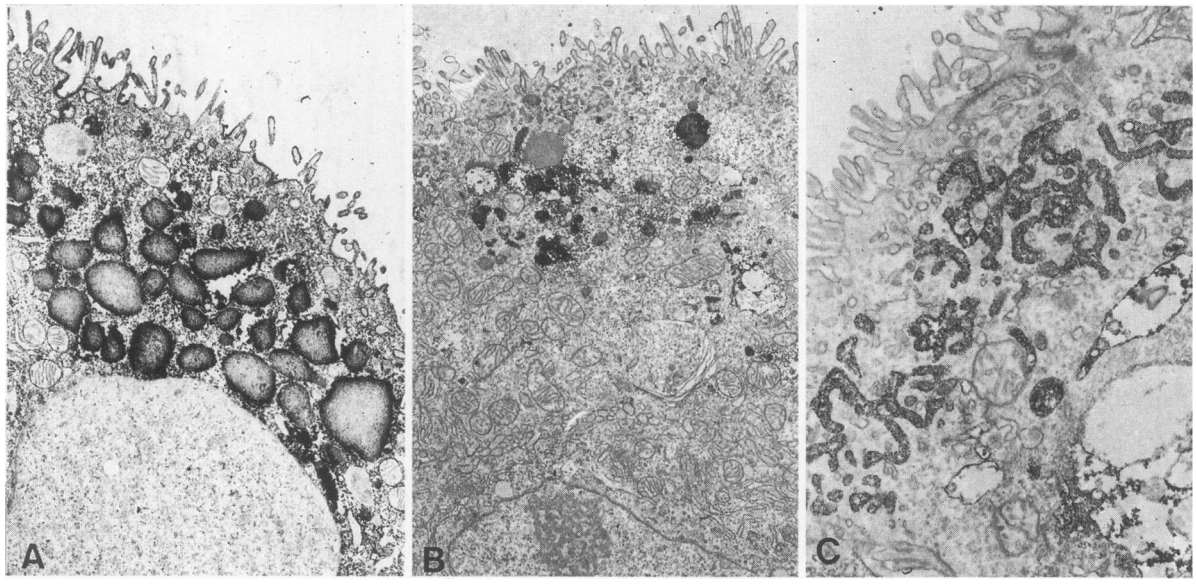
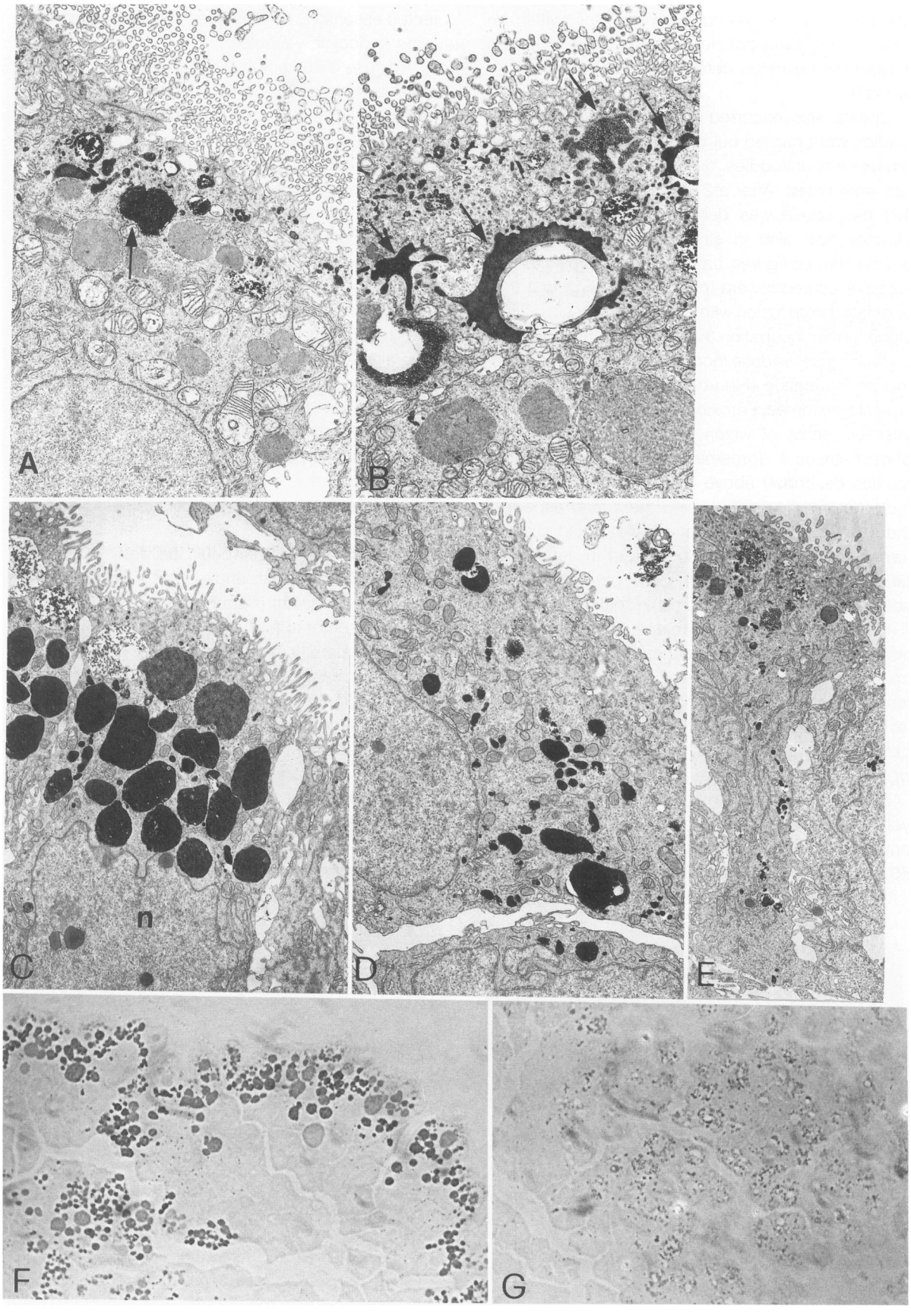


Figure 3. *Immunolocalization of rat IgG in epithelial cells of yolk sac cultured under control conditions* (A) *or in the presence of antibodies to gp280* (B, C) *during the 48-hour culture period. Note localization of rat IgG in lysosomes under control conditions* (A) *and in a large number of small vesicles in cultures exposed to antibody* (B). *In some instances rat IgG can be detected in a subapical tubular network* (C). *Original magnifications: A and B, ×3000; C, ×7000.*

Figure 4. *Detection of peroxidase in epithelial cells of yolk sacs cultured under control conditions* (A, C, F) *or in the presence of antibody to gp280* (B, D, E, G) *during the 48-hour culture period and exposed to peroxidase for 2 minutes* (A, B) *or 60 minutes* (C, D, E, F, G). *After a 2-minute incubation, under both conditions, reaction product is detected in numerous small subapical vesicles and in larger endocytic vacuoles (arrows). In cultures exposed to antibody to gp280* (B) *peroxidase is also detected in irregular-shaped structures (arrowheads). Note under both conditions the presence of unstained lysosomes. After a 60-minute incubation, peroxidase is essentially detected in large lysosomes in control cultures* (C); *in anti-gp280-exposed cultures* (D) *it is found in small-sized lysosomes, in large endocytic vacuoles, and in small vesicles along the lateral aspect of the cells* (E). *In semithin sections* (F, G) *note that peroxidase-containing vesicles are distinctly larger in control* (F) *than in anti-gp280 antibody-exposed cultures* (G). *Original magnifications: A–E, ×3000; F and G, ×40.*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der as well as in large endocytic vacuoles. Within the next minutes it was transferred to lysosomes in which it was overwhelmingly detected after 60 minutes (Figure 4C).

Uptake also occurred very rapidly when similar studies were carried out on yolk sac cultured in the presence of antibodies, but considerable abnormalities were noted. After a 2-minute incubation (Figure 4B) peroxidase was detected in numerous small vesicles and also in striking crescent, stellar, or puzzle-shaped figures. Large endocytic vesicles with irregular contours were found, suggesting that they originated from fusion with other vesicular structures. Upon further incubation the number of large vesicles containing peroxidase increased but even after a 60-minute incubation (Figure 4D) there were very few typical lysosomes. Peroxidase was detected in small vesicles, some of which probably contained acid phosphatases in agreement with the morphological studies described above. Peroxidase was also detected in small vesicles lining the lateral aspect of the cell (Figure 4E) in a pattern that could suggest transcytosis, and was seldom found in control cultures. This lack of accumulation of peroxidase in lysosomes was obvious on semithin sections (Figure 4, F and G), which could be subjected to morphoquantitative analysis. The area occupied by peroxidase-containing vesicles in yolk sac epithelial cells of cultures exposed to antibody to gp280 was 7.1% (n = 7), ie, significantly lower ($P < 0.001$) than in either control cultures (16.1%, n = 9) or cultures exposed to anti-gp330 antibody (18.6%, n = 7).

Internalization of peroxidase was also studied in yolk sacs cultured for 48 hours in the presence of antibodies to gp330 and exposed to peroxidase for 60 minutes. As shown in Figure 5 peroxidase was detected essentially in lysosomes and to some extent in large endocytic vacuoles.

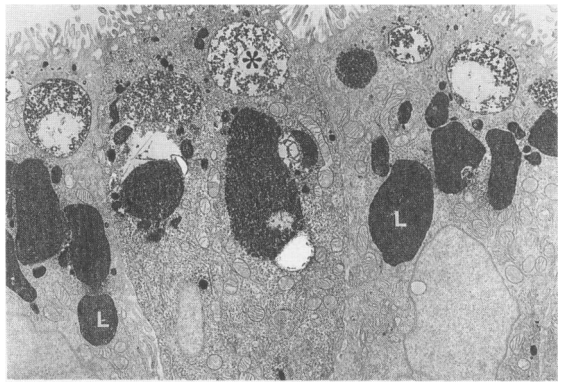

Figure 5. *Detection of peroxidase in epithelial cells of yolk sac cultured in the presence of antibody to gp330 during the 48-hour culture period and exposed to peroxidase for 60 minutes. Note presence of peroxidase in lysosomes (L) and to some extent in large endocytic vacuoles (*). Original magnification: ×3000.*

Because it was noted that culture in the presence of antibody to gp280 for 48 hours decreased growth of the yolk sac, additional experiments were carried out on embryos cultured under control conditions for 42 hours and in the presence of antibody to gp280 for 6 hours before exposure to peroxidase for 5 and 20 minutes. Observations made in control cultures in the latter incubation conditions were comparable to those reported above, as shown after 5 minutes in Figure 6A. In the presence of antibody to gp280, under conditions in which development of the yolk sac was normal, intracellular transit of peroxidase was still markedly altered. After a 5-minute exposure (Figure 6B) peroxidase was essentially localized to small apical vesicles both in the crescent/stellar type of formations described above and in an expanded network of dense apical tubules located immediately beneath the brush border. In some instances, tubules were directly connected to the crescent formations. When incubation was carried out further for 20 minutes, some of the peroxidase could be detected in lysosomes, but the vesicles and tubules described above remained prominent (data not shown).

In an attempt to further analyze the traffic of internalized material, experiments were carried out in which yolk sacs were exposed to peroxidase for 3 minutes and chased for an additional 20 minutes. Under control conditions, as shown in Figure 7A, peroxidase, initially present in endosomes and a few lysosomes, was rapidly transferred to lysosomes. In contrast, in cultures exposed to antibody to gp280, (Figure 7B and C) after the 20-minute chase peroxidase was still predominantly found in small vesicles of the early endocytic compartments. An intermediate pattern was observed in the presence of antibodies to gp330 (Figure 7D); peroxidase was efficiently transferred to lysosomes, but some of the early compartments of endocytosis remained labeled.

Discussion

The yolk sac is a complex structure which, in the rat, provides nutritive requirements for the embryo between the blastocyst stage and the time when the allantois becomes vascularized.[27] It is composed of a highly protein-permeable parietal layer and a visceral layer of epithelial cells characterized by a well-developed brush border, the presence of a large number of small and large endocytic vesicles, numerous lysosomes, and a network of dense apical tubules involved in membrane recycling.[28–33] These

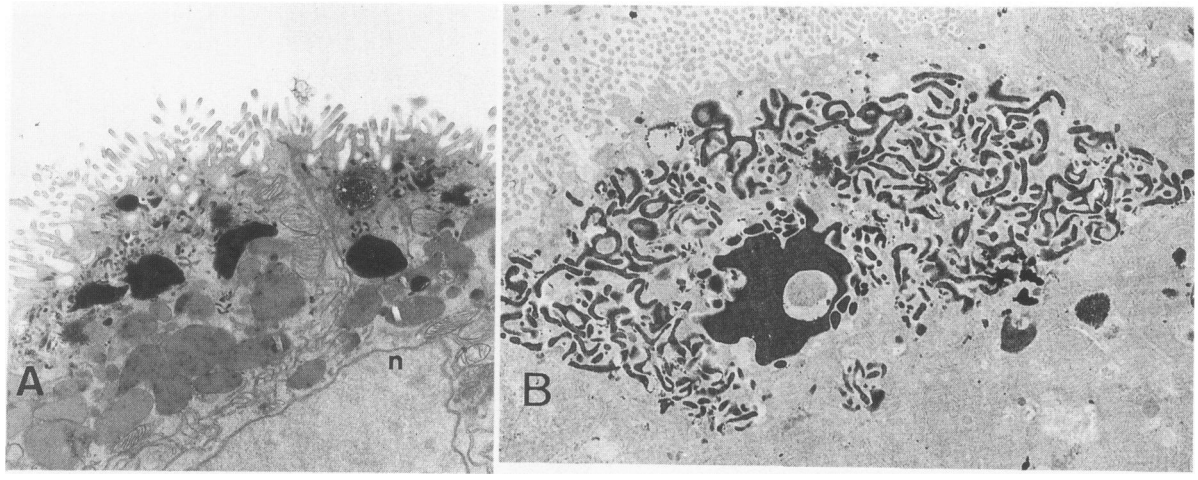

Figure 6. *Detection of peroxidase in epithelial cells of yolk sac cultured under control conditions* (**A**) *or in the presence of antibodies to gp280* (**B**) *during the final 6 hours of culture. Cultures were exposed to peroxidase for 5 minutes. Note that in antibody-exposed cultures, peroxidase is detected at 5 minutes in vesicles of abnormal shape and in tubular structures. Original magnification: ×4400.*

structural characteristics are associated with a high level of endocytosis, and[6] the amino acids used for embryonic development are essentially derived from internalized maternal proteins that are degraded. It is generally considered that teratogenic agents interfere at various levels with this sequence, resulting in a decreased flow of amino acids to the embryo, ultimately responsible for the induction of fetal malformations. In the case of trypan blue[9] or teratogenic antibodies it has been proposed, on the basis of decreased accumulation of fluid phase markers, that the primary defect was a decreased uptake of maternal proteins, and the presence of transient alterations of the apical pole[34] has been taken as evidence in favor of this hypothesis. On the other hand, inhibitors of lysosomal proteases presumably act later along the endocytic pathway by inhibiting breakdown of internalized proteins.[35–37] It is conceivable that a defect in the intracellular transfer of internalized material to the lysosomal compartment could also result in a decreased net flow of amino acids to the embryo.[8] Such a possibility is compatible with morphological abnormalities of visceral epithelial cells reported *in vivo* after injection of antibodies[38] including the presence of large numbers of electron-lucent vesicles and decreased numbers of lysosomes.

In this context, the object of this study was to analyze the effects of antibodies to a 280-kd coated pit protein, previously shown to be a target of teratogenic antibodies, on the endocytic properties of the visceral yolk sac. For this purpose we used *in vitro* cultures of embryos explanted at day 10, a system in which the visceral layer of the yolk sac conserves its polarity and constitutes the interface between the fetus and the culture medium, thus allowing study of some of its transport functions.

Our quantitative results showing that the amount of material taken up by fluid phase endocytosis was reduced by 40% are in agreement with data previously published using polyclonal[10] or monoclonal teratogenic antibodies.[11] However, in addition, our morphological results indicate abnormalities of the intracellular transit of internalized proteins. We confirmed on yolk sacs cultured for 48 hours in the presence of anti-gp280 antibodies that lysosomes were reduced in number and often of abnormal shape and size. Furthermore, staining for rat IgG clearly showed that antibodies to gp280 impeded the accumulation of maternal protein in lysosomes.

The early sequence of endocytosis could be further studied by adding peroxidase for 2 to 60 minutes at the end of cultures exposed to antibody for 48 or only 6 hours so as to obviate the consequences of gross underdevelopment of the yolk sac. Under both experimental conditions the antibody dramatically altered the transfer of peroxidase to lysosomes. The main abnormalities appeared to be localized in the early endocytic compartment visualized 2 to 5 minutes after incubation with peroxidase. They were characterized by a marked expansion of the dense apical tubular system and the presence of large stellar or crescent-shaped formations often prolonged by tubular extensions, which could represent small endocytic vacuoles/primary endosomes previously shown to be connected to dense apical tubules.[39] These formations were particularly prominent in cultures only exposed to antibody for 6 hours and remained partly labeled even after a 20-minute chase

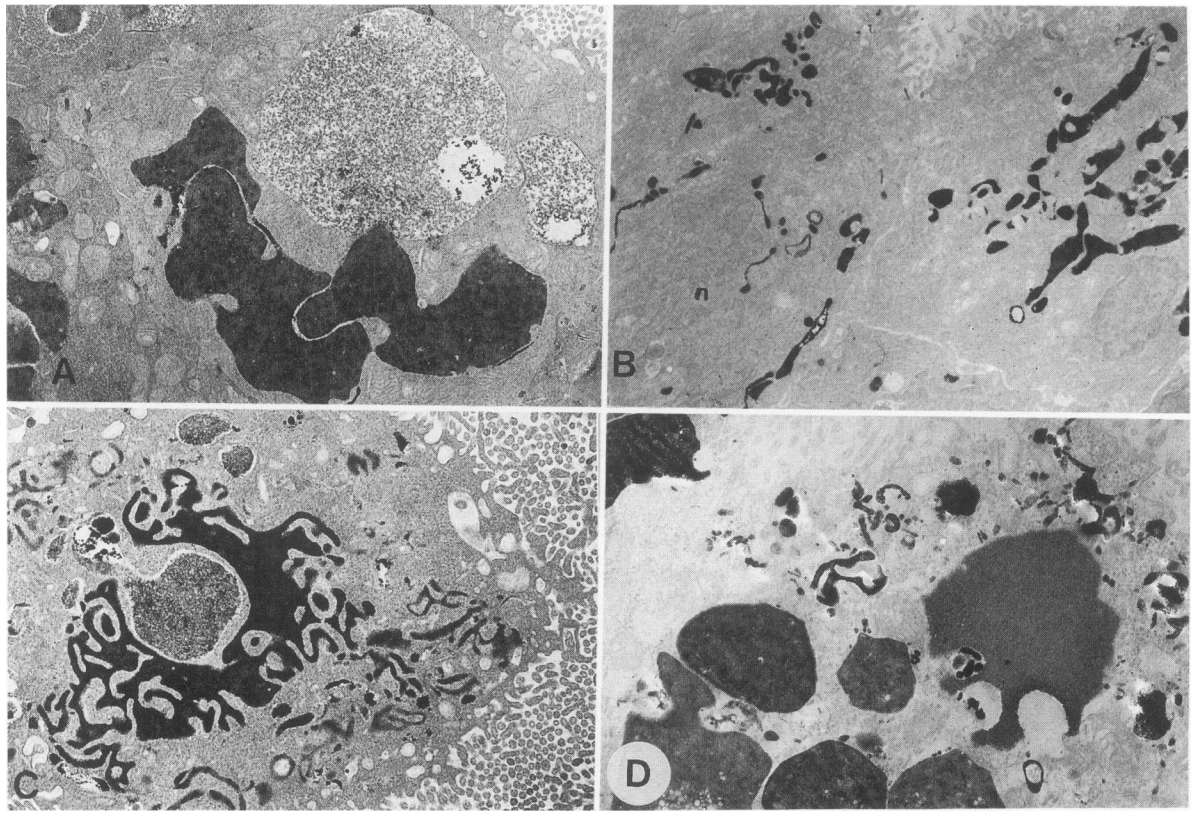

Figure 7. *Detection of peroxidase in epithelial cells of yolk sac cultured under control conditions* (**A**) *or in the presence of antibodies to gp280* (**B, C**) *or in the presence of antibodies to gp330* (**D**) *during the final 6 hours of culture. Cultures were exposed to peroxidase for 3 minutes and chased in the absence of peroxidase for 20 minutes. In control conditions peroxidase is only found in typical large lysosomes; in cultures exposed to antibody to gp280 it is detectable in smaller-sized vesicles and in some of the tubulovesicular structures that remain labeled. In the presence of anti-gp330 antibodies peroxidase is essentially localized in typical lysosomes as well as in smaller-sized vesicles. Original magnifications:* **A** *and* **B**, *×3000;* **C** *and* **D**, *×4400.*

in peroxidase-free medium, indicating an abnormal transfer to the late endocytic compartment. On the other hand, the formation of endocytic vesicles, ie, the initial internalization phase, was not quantitatively decreased by the antibody, since after a 2-minute exposure to peroxidase the numbers of small apical vesicles containing peroxidase were equivalent in control and antibody-exposed cultures.

The mechanisms involved in the effect of the anti-gp280 antibodies must still remain speculative. Taken together our observations, and in particular the expansion of the dense apical tubule network, suggest that anti-gp280 antibodies alter membrane recycling, which is the main function of this system.[28,40,41] It is likely that, after binding to gp280 expressed on the plasma membrane they act at least in part by altering the trafficking of their target protein, which, through trapping in a subcellular compartment and perhaps direct antibody binding to functional sites, becomes unable to perform its physiological role. Such a mode of action has been reported in tissue culture systems for antibodies specific for various components of the endocytic pathway including the mannose-6-phosphate[42,43] and the transferrin receptors.[44] Also,[45] cross-linking of membrane proteins by biotinylation of cell surface proteins followed by culture in the presence of avidin-induced abnormal primary endosomes, which increased in size and diminished transfer of peroxidase to lysosomes.

In all instances the modifications described are minor compared with the gross abnormalities reported here with antibodies to gp280 and in fact more closely approximate those induced in this study by antibodies to gp330, which as previously demonstrated[14] were unable to induce fetal malformations. This suggests that the effect of antibodies to gp280 is not only related to the formation of antigen antibody complexes that would alter the recycling of proteins but probably to the blocking of its biological properties.

One of the possibilities to be considered is that gp280 plays a key role in fusion and targeting of endocytic vesicles in some tissues. An alternative explanation depends on the binding properties of gp280, which could be a receptor for a ligand(s) yet to be determined. This hypothesis is supported by its similarities in distribution, immunoreactivity and bio-

chemical properties with gp330, which has been shown to be a multivalent binding protein.[46–49] It is thus possible that gp280 might be a polyvalent receptor involved in the transfer of large amounts of proteins from the external medium to the lysosomes.

We conclude that the main action of anti-gp280 antibodies is through altered trafficking of the target protein in the early endocytic compartment. Our findings expand observations of altered intracellular compartments as well as surface modifications induced by the antibodies. The decreased accumulation of fluid phase tracers previously reported was also found in our system and may be the consequence of the massive disruption of intracellular traffic.

Acknowledgments

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