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THE EXPRESSION OF HLA-G ANTIGEN IN DIFFERENT HUMAN TISSUES AND PLACENTAS AT DIFFERENT STAGES OF PREGNANCY

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Abstract: The distribution of HLA-G antigens in human tissues including placents was examined by immunohistochemical methods, using monoclonal antibody (Mab) specific to HLA-G antigen (87 G). Comparatively, class I specific Mab, W 6/32 was also employed for the detection of the antigen. W 6/32 reacted with a wide variety of cells and tissues throughout the body, whereas Mab 87 G did not react at all with these W 6/32 reactive cells except for those of placental tissues. Mab 87 G reacted with extravillous cytotrophoblast cells such as cytotrophoblastic cell columns, cytotrophoblastic shell and invasive cytotrophoblast but not other parts of placentas throughout the pregnant periods. Essentially the same results were obtained with W 6/32 staining in placentas. These results indicate that HLA-G antigens are exclusively expressed in extravillous cytotrophoblast cells although we cannot rule out the possibility that undetectable amounts of the HLA-G antigen or its isoforms which cannot be recognized by the Mabs used in this study are synthesized in some other tissues.

Index Terms

HLA class I, HLA-G, trophoblast, monoclonal antibody, histochemistry

INTRODUCTION

HLA-G is one of the nonclassical HLA class I genes discovered by Geraghty et al.[1~4]. Perhaps, since this discovery, the most significant recent finding about nonclassical antigens is that HLA-G is expressed in cytotrophoblast cells of the placenta[5~7]. This fact and the observation that HLA-G is not polymorphic, led to the hypothesis that HLA-G may play a fundamental role in protecting the placenta from a maternal immune response.

Wei and Orr demonstrated that HLA-G transcript was expressed in placenta but not in skin, liver, fetal thymus and lymphatic cells by RNase protection assay, suggesting that its expression is restricted to the placenta[7]. On the other hand, a low amount of HLA-G messenger RNA (mRNA) was detected in other tissues such as eye tissue, fetal thymus[8], spermatozoa[9], fetal liver[10] and skin[11] by means of the polymerase chain reaction method. Thus, the expression of transcript of HLA-G gene is not restricted to the placenta but it appears to be expressed in so-called immune privileged tissues, some fetal tissues and skin. However, at present, there is no direct evidence as to the expression of HLA-G antigen in these tissues as well as the placenta because no anti HLA-G antibodies were available.

Recently, several studies have been carried out to localize HLA-G mRNA in the placenta

using antisense HLA-G RNA probe[7,12~16]. On the other hand, the expression and localization of HLA-G antigen in the placenta has been deduced from the immunohistochemical experiments using anti HLA class I antibody, W 6/32 since several lines of data suggest that the majority, but not all, of the HLA class I antigen expressed in the placenta and recognized by W 6/32 is encoded by the HLA-G locus[13,17]. However, reported results were contradictory to each other on the distribution of mRNA and its translated product HLA-G antigen.

The purpose of the present study was to obtain exact information as to the distribution of HLA-G antigen in different human tissues as well as the placenta, at different stages of pregnancy by using the monoclonal antigbody against HLA-G antigen (Mab 87G) recently isolated by Gereghty et al. (in preparation).

MATERIALS AND METHODS

Tissue specimen

Most of the normal tissues other than placentas were obtained at forensic autopsies. The age of tissue donors ranged from stillborn babies to an 84-year-old female. Some tissues were also taken from a 58-year-old female at a pathological autopsy. The uterine myometrium, cervix, endometrium and hydatidiform mole were obtained at gynecological operations. Fresh chorions and placentas from the first trimester (5 to 11 week gestation) were obtained at abortions (19 cases) or by dilation and curettage (D & C). Nine samples of placentas from the second trimester (12 to 27 week gestation) and ten samples from the third trimester (28 to 40 week gestation) were obtained at delivery. The placentas from the second and third trimesters were also obtained at forensic autopsies.

These tissues were obtained after getting informed consent.

Monoclonal antibody 87 G (Mab 87G)

Anti HLA-G monoclonal antibody was isolated using a procedure essentially similar to that described by Hammerling et al.[18], using a transfected cell line rather than skin grafts. A detailed description will be published elsewhere (Ishitani and Geraghty, in preparation). Briefly, mouse cells transfected with HLA-G and human β2-microglobulin were used to immunize mice transgenic for HLA-B 27 and human β2-microglobulin. After a series of immunizations, spleen cells were fused with a myeloma cell line and cloned. The hybridomas were screened by staining human cells expressing HLA-G and analyzing by fluorescent cell sorting. The monoclonal antibody 87 G isolated by this method was subjected to analysis for its crossreactivity with HLA-A, B and C allels and for its reactivity with HLA-G. This analysis consists of four levels : 1)microcytotoxicity tests using over sixty cells from the Tenth International Workshop representing nearly all the known HLA class I specificities ; 2)FACS analysis using 26 cell lines from the Workshop covering most of the specificities ; 3)FACS analysis using six-LCLs of diverse HLA class I types transfected with the HLA-G gene in a comparative analysis with their untransfected counterparts ; 4)FACS analysis with choriocarcinoma cell line JEG-3 which expresses HLA-G on the surface. These analyses demonstrated that Mab 87 G reacted with HLA-G specifically and showed no significant crossreactivity with any HLA-A, -B, -C, alleles.

Other antibodies

Anti HLA class I antibody (W 6/32) and anti human cytokeratin antibody (CAM 5.2) were purchased from DAKO (Denmark) and Becton Dickinson (U. S. A.), respectively. CAM 5.2 was used for discriminating trophoblast cells from other cells[19].

Immunoperoxidase staining

The reactivity of these antibodies was investigated on frozen sections, because routine formalin fixation of tissues resulted in the disappearance of antigenicity recognized by Mabs used in this study. Tissues were cut about 0.5 cm^3, embedded in Tissue Tek II OCT compound (Miles, U. S. A.) and snap frozen. Frozen sections were cut about 6 μm by cryostat, air dried, and fixed in cold acetone for 10 min. After washing with 0.01 M phosphate-buffered saline (PBS) for 15 min, the sections were treated with distillated water containing 0.3 % hydrogen peroxide and 0.1 % sodium azide for 10 min, to abolish endogenous peroxidase activity according to the method of Li et al.[20]. Then, the sections were immersed in 10 % normal rabbit serum which is supplied as a blocking reagent of HISTOFINE SAB-PO(M) kit (Nichirei, Japan), for 15 min, in order to reduce nonspecific adsorption of the secondary anthbody. Following wash with 0.01 M PBS, the sections were incubated in the solution containing primary antibodies for 60 min at 37°C. The primary antibody, Mab 87 G, was used at a concentration of 10 μg/ml in 10 % normal rabbit serum. Both W 6/32 and CAM 5.2 were used at 25 μg/ml. Control sections were immersed in the solution of nonimmune mouse ascites (25 μg/ml, BALB/c control ascites fluid) (Cedarlane Laboratories Limitied, U. S. A.), and in solution without the primary antibody. After that, tissue sites reactive with these antibodies were visualized by streptavidine -biotin-peroxidase complex method, by using a HISTOFINE SAB-PO(M) kit with 3, 3′-Diaminobenzidine, tetrahydrochloride as a color development reagent. Finally, tissue sections were counterstained with haematoxyline.

RESULTS

Reactivity of the antibody with different human organs and tissues

Table 1 summarizes the results obtained by staining with W 6/32 and Mab87G in human tissues including placenta. The reactivity of W 6/32 with these tissues was quite different from that of Mab87G. W 6/32 reacted with a wide variety of cells and tissues throughout the body. In particular, the capillary endothelium and fibroblasts in all the tissues examined were stained positively with W 6/32. Except for certain cells of the genital system and pancreatic Langerhans cells which were negative with W 6/32, it exhibited similar staining properties to those of the PA 2.6 antibody[21]. In contrast to such a wide distribution of reactivity with W 6/32 (Fig. 1 and 3), Mab 87G could not react with any cells (Fig. 2 and 4) except for placental tissues. Since HLA-G antigen was detected by Mab87G in the placenta taken from the longest postmortem cadaver available (about 30 hours), it is unlikely that the absence of reactive sites with 87G in other tissues was due to the postmortem changes of these tissues.

Table 1. Expression of HLA class I and HLA-G antigen in different human organs and tissues

Tissue	W 6/32	Mab 87 G
Endocrine system :		
Thyroid (follicular)	−	−
Pancreatic islets of Langerhans	−	−
Adrenal		
Cortical zone	+~++	−
Medulla	+~++	−
Gastorointestinal tract :		
Epithelium of		
Tongue	+	−
Stomach	±	−
Ileum	±	−
colon	±	−
Gall bladder	+	−
Liver		
Sinusoidal lining cells	+	−
Hepatocytes	±	−
Salivary gland :		
Parotid gland (acinus, duct)	−	−
Submandibular gland (acinus, duct)	−	−
Sublingual gland (acinus, duct)	−	−
Respiratory and cardiovascular system :		
Alveolar epithelium of lung	+	−
Heart	+	−
Myocardium	±	−
Endothelium	±	−
Capillaries	++	−
Larger vessels	++	−
Urogenital system :		
Kidney glomeruli		
(endothelium, mesangium)	+~++	−
Kidney tubules	+~++	−
Epithelium of		
Ureter	+	−
Bladder	++	−
Genital system :		
Testis		
Germcell line, Sertoli cell	−	−
Leydig cell	+~++	−
Spermatozoa	−	−
Ovary		
Cortical zone	−	−
Medulla	−	−
Uterus		
Cervix	±	−
Myometrium	±	−
Endometrium	+~++	−
Epithelium of uterine tube	−	−
Mammary gland	−	−
Lymphatic system :		
Thymus		
Cortical zone	−	−
Medulla	++	−
Spleen	+	−

Tissue	W 6/32	Mab 87 G
Nervous system :		
Central		
Neurones	−	−
Dura	+	−
Sensory system :		
eye		
Choroid	+	−
Retina	+	−
Skin :	+~++	−
Muscle :		
Skeletal	−~±	−
Smooth	±~+	−
Fibroblast :	+~++	−

Table 2. Localization of HLA antigens in human placentas at different stages of pregnancy

Tissue	W 6/32	Mab 87 G
First trimester :		
Chorionic villi		
Syncytiotrophoblast	−	−
Cytotrophoblast	−	−
Extravillous trophoblast		
Cytotrophoblastic cell columns	+	+
Cytotrophoblastic shell	+	+
Invasive trophoblast	+	+
Mesencymal cell (immature Stroma)	+	−
Decidua	+	−
Second trimester :		
Chorionic villi		
Syncytiotrophoblast	−	−
Cytotrophoblast	−	−
Extravillous trophoblast		
Cytotrophoblastic cell columns	+	+
Invasive trophoblast	+	+
Stroma	+	−
Decidua	+	−
Third trimester :		
Choriomic villi		
Syncytiotrophoblast	−	−
Extra-villous trophoblast		
Invasive trophoblast	+	+
Stroma	+	−
Decidua	+	−

Reactivity with human placentas at different stages of pregnancy

The tissue sites reactive with W 6/32 and Mab87G were essentially the same in the placenta (Table 2). In the first trimester, both of these antigens reacted with cytotrophoblastic cell columns, cytotrophoblastic shell and invasive cytotrophoblast, but not with the chorionic villous cytotrophoblast and syncytiotrophoblast (Fig. 5 and 6).

In the second trimester, cytotrophoblastic cell columns and invasive cytotrophoblast were also stained with these two antibodies. In this case too, they did not react with chorionic villous cytotrophoblast and syncytiotrophoblast. The structures corresponding to the cytotrophoblastic shell found in the first trimester disappeared in the second and third trimes-

ter.

Essentially the same results were obtained in the third trimester. Thus, both antibodies reacted with invasive cytotrophoblast but not with chorionic villous syncytiotrophoblast (Fig. 7 and 8). The structures corresponding to cytotrophobastic cell column and villous cytotrophoblast found in the first and second trimester were not detected in the third trimester. Cytotrophoblastic cells of hydatidiform mole were likewise stained with these Mabs (Fig. 9). W 6/32 reacted diffusely with mesenchymal cell (immature stroma) of the first trimester placenta and stroma of the second and third trimester placenta whereas Mab87G did not.

Usually these Mabs reacted with many of these extra villous trophoblast cells (80～70 %) in most of the cases examined. However, in one abortion case, Mab87G stained only a limited number of these cells (less than 10 %) in the placenta. In this case, W 6/32 reacted with most of the extravillous trophoblast and mesenchymal cell as in other cases.

DISCUSSION

In contrast to the ubiquitous expression of the classical HLA class I antigens as disclosed by the staining with W 6/32, the expression of HLA-G antigen was shown to be restricted to the placenta by immunohistochemical method using Mab87G which is specific to the antigen. Although mRNA of HLA-G was detected in some other tissues such as eye tissue, fetal thymus[8], spermatozoa[9], fetal liver[10] and skin[11], we failed to demonstrate the antigen in these tissues in the present study. As to the thymus and liver, the presence of mRNA has been reported to be restricted to the tissues in the early fetal stage, which were not included in the present study. At present, we can not determine whether the antigens are not synthesized due to certain posttranscriptional regulations or the content of the antigen is too low to be recognized by the Mab in these tissues. To clarify the problem, we need more extensive studies with sufficient numbers of samples, especially with early fetal tissues.

Since the reactive sites with W 6/32 corresponded well with those with the anti HLA-G antibody in placentas, its reactivity can be ascribed to the presence of the HLA-G antigen, confirming the previous suggestion that HLA class I antigens expressed in trophoblast cells do not correspond to classical HLA-A, B, C molecules[22]. Although Yelavarthi et al. demonstrated the presence of HLA-G mRNA in villous cytotrophoblast[13] while Lata et al. found it in syncytiotrophoblast[14] by using in situ hybridization techniques, we could not detect the antigen in these cells. According to Ishitani and Geraghty, three kinds of isoforms of HLA-G antigen, that is, HLA-G1, G2 and G3 proteins, appear to be expressed in the placenta[23]. Since Mab87G can hardly react with G-2 and G-3 proteins (preliminary observation) as W 6/32 and mRNA of these proteins have untranslated regions which can be detected by RNA probe containing antisense sequence of this region, we can not neglect the possibility that G-2 and/or G-3 but not G-1 proteins are preferentially and selectively synthesized in these tissue sites. Of course, the present results may also be explained by assuming that the antigen is synthesized only in an undetectable amount or not produced at all in these cells. Further study is required for clarifying the problem, using Mabs exhibiting different specificity for isoforms of HLA-G antigens.

Since cytotrphoblastic cell columns, cytotrophoblastic shell and invasive cytotrophoblast

were placed on the border between the tissues originating from the mother and her fetus, specific localization of the HLA-G antigen in these tissue sites further substantiates the assumption that the HLA-G antigen plays an essential role in the immunologic processes during pregnancy. In this respect, it is interesting to note the fact that in one abortion case, Mab87G reacted faintly with only a small number of extra villous cells. Thus, in this case, the decrease in the contents or modification of HLA-G antigen is supposed to be responsible for intrauterine fetal death.

By using the Mab specific to HLA-G antigen, the results of the present study provide confirmative evidences that HLA-G antigen is exclusively expressed in extravillous cytotrophoblast cells in placentas. Further study is required for elucidating more precise distribution of HLA-G antigen, especially its isoforms in different human tissues as well as placentas.

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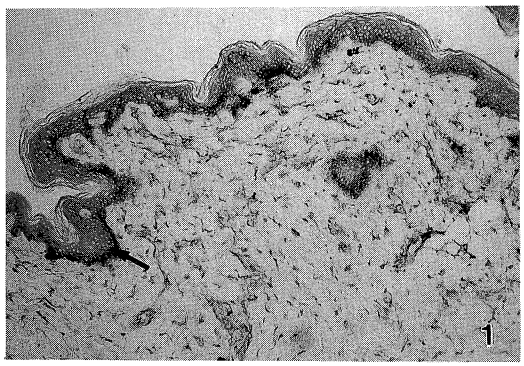

Fig. 1. W 6/32 staining of the skin. Stratumspinosum (arrow) and connective tissue are stained with W 6/32. (×100)

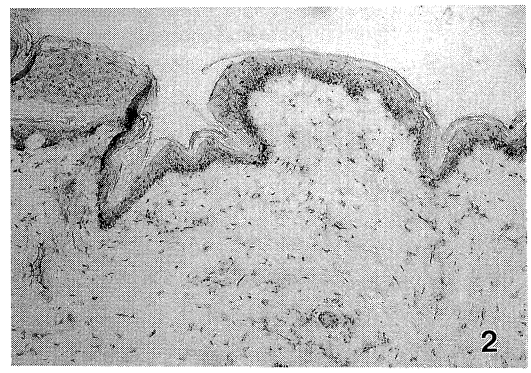

Fig. 2. Mab87G staining of the skin. No staining is seen. (×100)

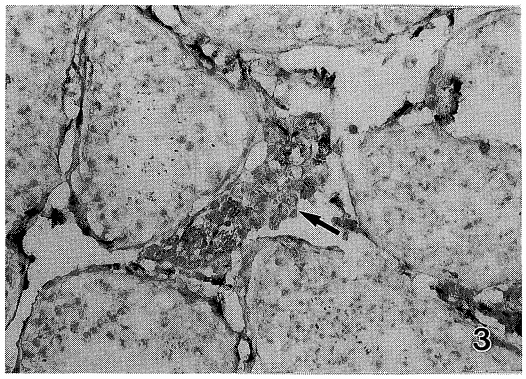

Fig. 3. W 6/32 staining of the testis. W 6/32 reacts with Leydig cells (arrow) but not with Sertoli cells and spermatozoa. (×200)

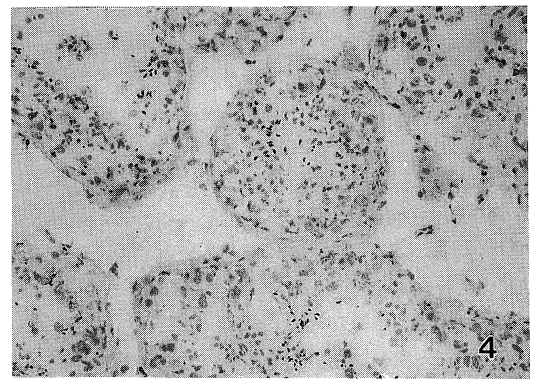

Fig. 4. Mab87G staining of the testis. No staining is seen. (×100)

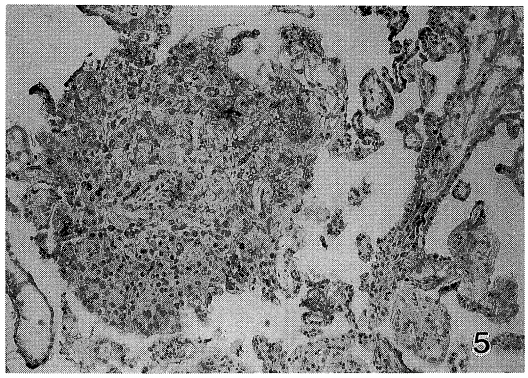

Fig. 5. W 6/32 staining of first trimester placenta. Cytotrophoblastic cell column and villous mesencymal cells are positive with W 6/32. (×200)

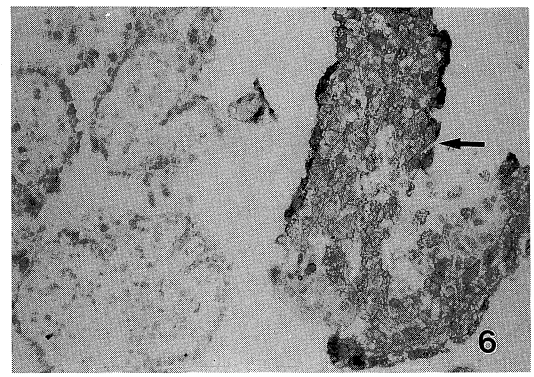

Fig. 6. Mab87G staining of first trimester placenta. Only cytotrophoblastic cell column (arrow) are stained with Mab87G. Villous trophoblast and mesencymal cells are not stained with the Mab. (×200)

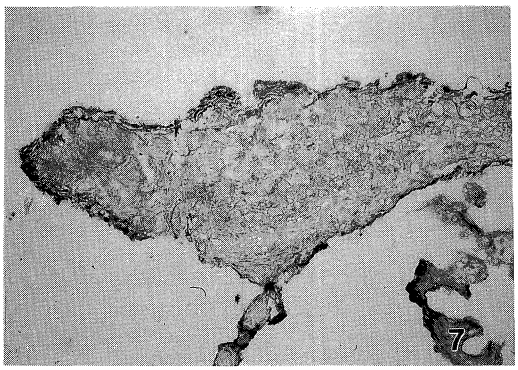

Fig. 7. W 6/32 staining of third trimester placenta. Both decidua and villous stroma are stained with W 6/32. (×100)

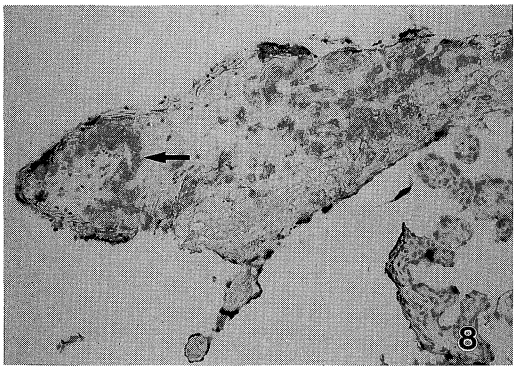

Fig. 8. Mab87G staining of third trimester placenta. The Mab reacts with cytotrophoblast cells invasting decidua (arrow), but not with other parts of placenta. (×100)

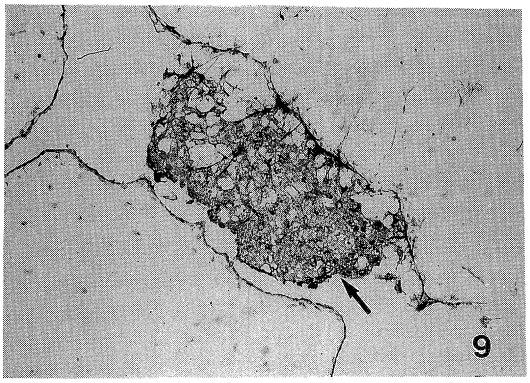

Fig. 9. Mab87G staining of hydatidiform mole. The Mab reacts only with cytotrophoblastic cell clusters as found in cytotrophoblastic cell columns (arrow). (×100)