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21. This work was supported by NIH grants CA-31867, CA-09215, and program project grant CA-13525; the Aaron Silvera fund; and the Andres Soriano cancer research fund. We thank Dr. R. E. Meyn for advice on the fluorescent DNA assay.

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28 June 1983; accepted 29 September 1983

Human Trophoblast-Lymphocyte Cross-Reactive (TLX) Antigens Define a New Alloantigen System

Abstract. *Antisera to human syncytiotrophoblast microvillous cell surface membranes from different placentas are cytotoxic for lymphocytes from some people but not others, demonstrating the presence of allotypic trophoblast-lymphocyte cross-reactive (TLX) antigens. Exploratory principal components factor analysis, performed on limited data consisting of 300 cytotoxic reactions produced by ten separate trophoblast antisera on a panel of lymphocytes from 30 random donors, suggested the presence of three distinct TLX antigen groupings. It is proposed that such TLX alloantigens are central in establishing maternal recognition and protection of the blastocyst, and that lack of recognition results in implantation failure and spontaneous abortion. These findings are compatible with contemporary results of immunotherapy to prevent recurrent spontaneous abortions, and their implications extend to other conditions of allogeneic coexistence, such as organ transplantation and the tumor-host relationship.*

During human pregnancy, the principal anatomical interfaces between maternal and extraembryonic tissues are formed by the trophoblast (*1, 2*). Immunohistological studies of these human placental villous trophoblasts show none of the major histocompatibility complex (HLA) (*3, 4*) or ABO blood group (*5*) antigens; the membranes do, however, express two unique groups of species-specific cell-surface trophoblast antigens (TA) that have been designated TA1 and TA2 (*6*). Antisera defining the TA1 group are trophoblast-specific, yet they can functionally inhibit allogeneic recognition as shown by their ability to block the mixed lymphocyte culture (MLC) reaction (*7*). Antisera of TA2 specificity are cytotoxic for lymphocytes (*8*), indicating the presence of trophoblast-lymphocyte cross-reactive (TLX) antigens. We have proposed that these TA groups act in a hapten-carrier model of pregnancy (*6*), TA1 as the carrier and TA2 the hapten. TA1 can modulate cell-mediated immunity that would theoretically result within placentas from such a hapten-carrier system (*9*), and the need for stimulating maternal recognition reactions has been met by data showing the TA2 group of TLX antigens to be allotypic (*10*). We now report findings suggestive of a new alloantigen system for TLX as revealed by principal components factor analysis (*11, 12*) of the differential lymphocytotoxicity produced by trophoblast antisera with human peripheral blood lymphocytes (PBL). The results show that trophoblast antisera produce cytotoxic reaction patterns with PBL which are suggestive of three TLX antigen groups. Groups 1 and 3 were found to represent two independent factors, and group 2 was a combination of the reaction patterns produced by groups 1 and 3.

A principal components factor analysis with varimax rotations (*13*) was used in an exploratory manner for the purpose of providing a statistical organization of the cytotoxicity reactions of ten trophoblast antisera (A through J) with a panel of PBL from 30 donors. This analysis showed that the reaction patterns could be attributable to two factors (Fig. 1). The similarity of the reactions of five of the antisera (D, F, G, H, and I) with the 30 PBL donors is indicated by sizable factor loadings of these sera on a common factor (factor 1). Likewise, the similarity of the reactions of three sera (B, C, and E) with the 30 PBL donors is indicated by loadings on a second factor (factor 2). Sera D, F, G, H, and I had loadings of 0.68 or greater on factor 1 and 0.36 or less on factor 2 (Table 1), whereas sera B, C, and E had loadings of 0.60 or greater on factor 2 and 0.22 or less on factor 1. Sera A and J had approximately equal loadings on factors 1 and 2 (Table 1). These findings suggest the existence of three distinct TLX antigen groups in terms of their reactions with lymphocytes. The three reaction groupings are depicted in Fig. 2 in which the reactions

Table 1. Factor structure matrix showing the loadings on factors 1 and 2 for the lymphocytotoxic reaction patterns produced by ten antisera to trophoblasts.

Sera	Factor 1	Factor 2
A	0.62	0.62
B	0.18	0.68
C	0.22	0.86
D	0.81	0.36
E	−0.08	0.60
F	0.76	0.05
G	0.79	0.12
H	0.84	0.06
I	0.68	0.30
J	0.38	0.69

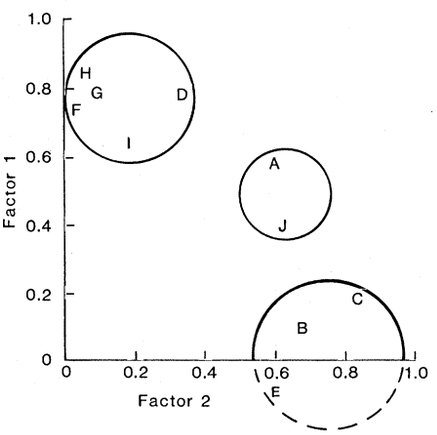


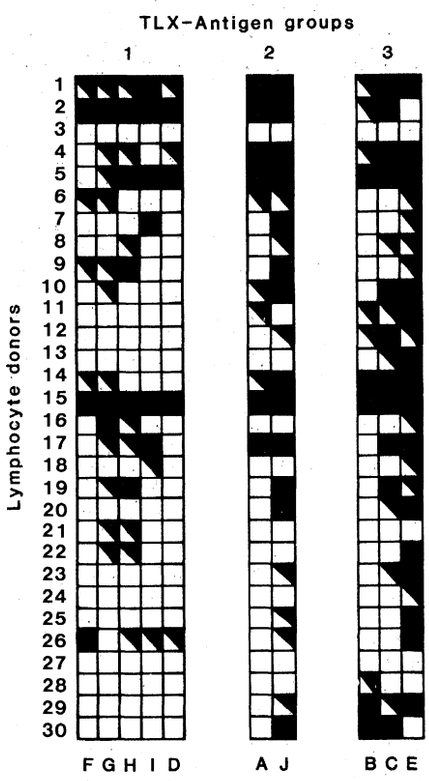
Fig. 1. Principal components factor analysis with varimax rotation of the cytotoxicity reaction patterns of ten antisera to trophoblasts provides evidence for three groups of TLX antigens. A similar factor structure with the same TLX antigen grouping was obtained with principal axis factor analysis with varimax rotation.

of the ten trophoblast antisera A through J with PBL are arranged according to the groupings given by factors 1 and 2. TLX group 1 is composed of sera D, F, G, H, and I that reflect factor 1, and TLX group 3 is made up of sera B, C, and E that reflect factor 2. TLX group 2 is comprised of sera A and J that reflect both factors 1 and 2, analogous to the AB blood group classification that reflects blood groups A and B. According to our conceptual model for TLX (Fig. 3), groups 1 and 3 are separate, whereas TLX group 2 consists of components from both TLX groups 1 and 3.

Statistical analysis of the pooled data has shown that TLX groups 1, 2, and 3 account for 63.1 percent of the total variance ascribed to the TLX system, leaving 36.9 percent due to unknown factors. However, because only ten trophoblast antisera were used in the study, and only 30 leukocyte donors were tested, there is no assurance either that the ten sera represented all existing TLX groups or that the 30 donors provided the opportunity for the demonstration of reaction patterns indicative of other TLX groups. Evidence in favor of this possibility is drawn from the observation that of the 30 different leukocyte donors tested, individuals 3 and 27 (Fig. 2) failed to react with any of the antisera. Regardless of the limitations of these data, a factor analysis approach would seem to provide a reasonable means of organizing this type of cytotoxicity data for the purpose of suggesting possible TLX antigen groupings.

Another and possibly more serious limitation of these data is that they necessarily do not include TLX combinations that are incompatible with normal embryonic growth and development. Indeed, we have hypothesized that too great a degree of TLX antigen similarity between a husband and his wife would fail to provoke the appropriate allotypic recognition responses of blastocystic trophoblast by the mother (*14*), resulting in a lack of blastocyst protection by blocking factors or suppressor cell production, thereby allowing conventional rejection reactions in the maternal uterus to terminate the pregnancy as a spontaneous abortion (*3*).

Statistical comparisons of the reaction patterns of the ten TLX antisera with HLA, A, B, C, and DR types of the 30 lymphocyte donors failed to show any significant correlations (*10*), and analyses of the antisera with lymphocytes from individuals known to be homozygous for one or more HLA alleles also produced negative findings. These results indicate that antiserum to TLX is not identifying HLA, but they are not incompatible with the idea that TLX is either in linkage disequilibrium with HLA or that TLX alloantigens may serve as a minor histocompatibility complex (*15*). Several investigators have shown that recurrent spontaneous abortions are associated in some couples with an increased sharing of HLA antigens between husband and wife (*16–18*). We have confirmed these observations in a well-defined group of primary spontaneous aborters (*19*) by showing an association between HLA and TLX antigens in which couples who share HLA are at higher risk to share TLX (*15*). Indeed, clinical studies designed to provide primary spontaneously aborting women with prophylactic TLX stimulation by infusion of HLA mismatched (that is, TLX disparate) leukocytes have been successful in maintaining pregnancies to term (*20*). These clinical results in conjunction with the present laboratory evidence obtained with the use of factor analysis methodology provide incentives for further immunogenetical studies of the TLX system in normal and abnormal reproduction, particularly including human primary and secondary recurrent spontaneous abortions (*21*). The role of TLX antigens as manifest in the materno-trophoblastic relationship may extend to other conditions of allogeneic coexistence, as in organ transplantation or tumor-host biology, and such implications are supported by the results of ongoing research in our laboratories.



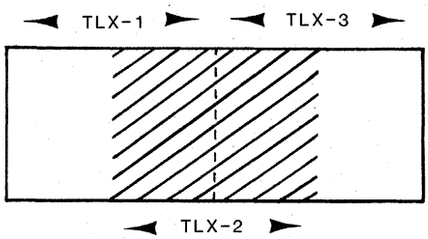


Fig. 2 (left). Three groups of lymphocytotoxic reaction patterns of ten (A to J) individually prepared antisera to trophoblasts with peripheral blood leukocytes taken from 30 random donors: □, < 20 percent killed; ◪, 50 percent killed; and ■, > 80 percent killed. Antisera were prepared by isolating syncytiotrophoblast membrane microvilli pellets from ten separate placentas by the technique of Smith *et al.* (*22*). Ten rabbits were immunized, each with one placental pellet as previously described (*7*). Antisera were heated at 56°C for 30 minutes, absorbed with pooled human erythrocytes until negative for microscopic agglutination, and passed through a solid-phase immunoabsorbent column of normal human sera (NHS) until negative by immunoelectrophoresis and gel diffusion against NHS. To assess cytotoxicity, we incubated 2 μl of each antisera in triplicate at room temperature for 1 hour and with 2 μl of cell suspension (2×10^6 cells per milliliter) from each PBL donor and then added 4 μl of rabbit complement without washing the cells. After 2 hours, trypan blue was added and the viability assessed. The dilutions of the antisera were initially determined by testing lymphocytes from ten donors at dilutions ranging from neat to 1:12,800. The working dilution for each antiserum was determined by using the highest dilution that continued to kill > 80 percent of at least one individual's cells. Fig. 3 (right). The expression of TLX group antigens can be represented by this model which shows that TLX group 1 and TLX group 3 are distinct and separate from each other. In contrast, TLX group 2 consists of significant amounts of TLX 1 and TLX 3.

In conclusion, the present laboratory evidence obtained with the use of factor analysis methodology provide incentives for further immunogenetical studies of the TLX system in normal and abnormal reproduction, particularly including human primary and secondary recurrent spontaneous abortions (*21*). The role of TLX antigens as manifest in the materno-trophoblastic relationship may extend to other conditions of allogeneic coexistence, as in organ transplantation or tumor-host biology, and such implications are supported by the results of ongoing research in our laboratories.

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23. Supported in part by the March of Dimes Birth Defects Foundation, the Human Embryology and Pregnancy Foundation, and the Luzern Medical Research Institute.

15 June 1983; accepted 30 August 1983

Dynorphin-A-(1–8) Is Contained Within Vasopressin Neurosecretory Vesicles in Rat Pituitary

Abstract. *Dynorphin-A-(1–8), an opioid peptide widely distributed in the rat central nervous system, is present in vasopressin-containing neurosecretory cells terminating in the neural lobe of the pituitary. Electron microscopic immunocytochemistry reveals that dynorphin-A-(1–8) is contained within the same neurosecretory vesicles as vasopressin and vasopressin-associated neurophysin in the neural lobe of the rat. The results indicate that dynorphin may be released in the pituitary concomitantly with vasopressin during the antidiuretic response.*

Dynorphin A was originally isolated from porcine pituitary and gut as an extremely potent 17-amino-acid opioid peptide with leucine-enkephalin ([Leu]-enkephalin) at its amino terminus (*1*). It is widely distributed in rat brain (*2*), and has been shown to be present within vasopressin-containing magnocellular neurons (*3*). The amino-terminal octapeptide dynorphin-A-(1–8) is the predominant form of dynorphin in rat brain (*4*). The messenger RNA sequence for the prodynorphin precursor has been determined by cloning and sequence analysis of complementary DNA (*5*). The precursor contains the sequence for a previously isolated 32-amino-acid peptide consisting of dynorphin A at the amino terminus and a related tridecapeptide (dynorphin B) at the carboxyl terminus (*6*). The precursor also contains the sequence for α-neo-endorphin, which explains the identical distributions by immunocytochemistry of dynorphin and α-neo-endorphin (*2*), and the approximately equimolar concentrations of dynorphin-A-(1–8) with α-neo-endorphin in all regions of rat brain (*4*). Immunoreactive dynorphin A and α-neo-endorphin are released in approximately equal amounts in a Ca^{2+}-dependent manner from isolated neurointermediate lobes of the pituitaries of developing postnatal rats (*7*). In view of the possible influence of opioid peptides on the release of vasopressin and oxytocin in the neurohypophysis (*8*), it is important to characterize the synthesis, processing, packaging, and release of dynorphin and related peptides in magnocellular neurosecretory cells. Dynorphin-like immunoreactivity in rat neurointermediate lobe homogenates migrates in sucrose density gradients at the same rate as vasopressin (*9*), which suggests that dynorphin may be located in vasopressin neurosecretory vesicles. [Met]Enkephalin or [Leu]enkephalin immunoreactivity (or both) has been located in neurosecretory vesicles in oxytocin or vasopressin axons (or both) in rat (*10*) and cat (*11*) neurohypophysis by electron microscopic (EM) immunocytochemistry. However, [Leu]enkephalin immunoreactivity has also been located by EM immunocytochemistry in fibers making synaptoid contacts with neurohypophyseal glial cells (pituicytes), but no evidence was found for the coexistence of the enkephalins with vasopressin or oxytocin in the same terminals (*12*).

We have been able to locate dynorphin-A-(1–8) on the EM level by using a recently developed postembedding immunostaining method that stains intensely without the use of etching or enzymatic treatment of the sections. In addition, the use of serial ultrathin sections has allowed us to unequivocally compare the intraaxonal distributions of dynorphin-A-(1–8), vasopressin, and oxytocin.

Adult heterozygous Brattleboro rats were perfused through the heart with 4 percent paraformaldehyde, 2 percent glutaraldehyde, 0.2 percent picric acid, and 0.1*M* sodium cacodylate (*p*H 6). The pituitaries were embedded in LR White

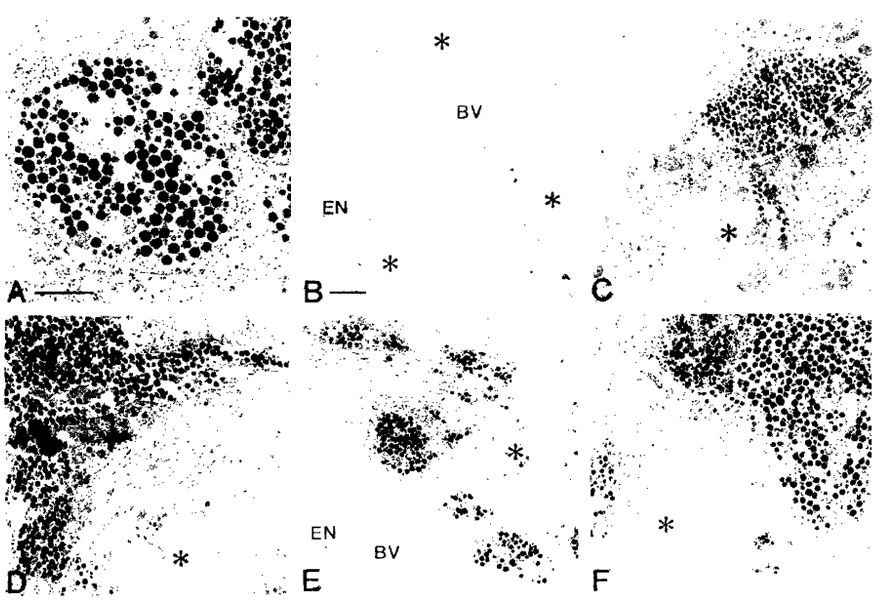

Fig. 1. Immunostaining of neurosecretory vesicles for dynorphin-A-(1–8) in the rat posterior pituitary. (A) Section stained with dynorphin-A-(1–8) antiserum. (B) Stained with dynorphin-A-(1–8) antiserum absorbed with dynorphin-A-(1–8). No staining is present. (C) Dynorphin-A-(1–8) antiserum absorbed with dynorphin-A-(1–13). Staining is partially inhibited. No inhibition of staining results from absorbing the dynorphin-A-(1–8) antiserum with [Leu]enkephalin (D), arginine vasopressin (E), or cholecystokinin (F). Asterisk, unlabeled axon. Abbreviations: *BV*, blood vessel; *PN*, pituicyte (glial) nucleus; *EN*, endothelial cell nucleus. Bars represent 1.0 μm; (C) to (F) are printed at the same magnification as (B).