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17β ESTRADIOL AFFECTS THE EXPRESSION OF GUINEA PIG BLOOD LEUKOCYTE MHC ANTIGENS

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Summary—Sex hormones have an effect on various immune responses but the mechanisms of action are unknown. One of these mechanisms might be a modification of expression of major histocompatibility complex (MHC) antigens in blood leucocytes. Estradiol-induced variations of the expression of guinea pig blood leukocytes MHC antigens (GPL-A) was studied. Class I and class II MHC antigens were detected by a sensitive rosetting method using specific alloimmune sera (AIS) and staphylococcal protein A-coated sheep red blood cells (SPA-SRBC) and evaluated by counting the number of bound SPA-SRBC per 100 cells. MHC antigens decreased after estrogen treatment. Estradiol modifies the expression of GPL-A antigens on the mononuclear cells including the Kurloff cells, which are involved in immunity or in a natural killer effect, but did not affect the expression of polymorphonuclear cells, ones which are not involved in immunity.

INTRODUCTION

Sex hormones control the regulation of different immune responses [1, 2] by interacting *in vivo* with a variety of cells. These hormones and, in particular estrogens, have been clearly shown to influence the major histocompatibility complex (MHC) on cells of the genital tract [3–7] and embryonic tissues [8] though no sex hormone–MHC antigen interactions have yet been demonstrated with blood leukocytes.

The guinea pig MHC or guinea pig leucocyte antigen complex (GPL-A) has been well documented since 1973 [9] and two classes of antigens have been described [10]: class I antigens (B1, B2, B3 or B4, S1)—these ubiquitous antigens correspond to mouse H2-D and H2-K antigens or to human HLA-A or HLA-B antigens; and class II antigens or immune associated antigens (Ia)—seven specificities have currently been defined (1, 2, 3, 4, 5, 6 and 7) and are present on immunocompetent cells (monocytes, macrophages, Langerhans cells, B- and activated T-lymphocytes). Class I and class II antigens are also present on some tumor cells including the L2C-N Leukemia [11]. It was reported that, unlike in mice or humans, most normal resting guinea pig T-cells and most thymocytes express Ia antigens [12]. Class II antigens were also described on several glandular epithelial cells including guinea pig mammary gland cells [13].

Guinea pig leukocytes include a specific and hormone-sensitive cell [14], the Kurloff cell. Repeated *in vivo* injections of estradiol induce a considerable increase in the number of Kurloff cells in the spleen, thymus and blood [15, 16]. This mononuclear cell (MN) is characterized by the presence of a unique intracytoplasmic inclusion rich in proteoglycans [17] and lysosomal enzymes [18]. It develops a natural killer activity [19].

This report demonstrates the hormonal modulation of GPL-A antigen expression on different blood leukocytes including Kurloff cells.

We characterized class I and class II antigens using a rosetting assay with alloimmune sera (AIS) and staphylococcal protein A-coated sheep red blood cells (SPA-SRBC) to detect GPL-A antigens [20], and compared

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Abbreviations: MHC, major histocompatibility complex; GPL-A, guinea pig leucocyte antigen complex; AIS, alloimmune serum; SPA-SRBC, staphylococcal protein A-coated sheep red blood cells; PBL, peripheral blood leukocytes; PMN, polymorphonuclear cells; MN, mononuclear cells.

their expression on peripheral blood leukocytes (PBL) from normal and estrogenized females. Hormone effect does not concern the polymorphonuclear cells (PMN) but concerns MN. This hormone effect might be a mechanism of action of sex hormones on various hetero- or auto-immune responses [21].

MATERIALS AND METHODS

Animals

Inbred female guinea pigs from 3 different strains were used: strain 2 (S.2) (GPL-A: B1, S1, Ia 2, 4, 5, 6) and strain 13 (S.13) (GPL-A: B1, S1, Ia 1, 3, 5, 6, 7) supplied by the Medical Research Council (London), and strain BIO-AD (BIO-AD) (GPL-A: B3, Ia 1, 3, 5, 6, 7) kindly donated by Professor A. L. De Weck (Bern, Switzerland). All were maintained in one of our departments. The animals were divided into two groups: normal and estrogenized animals (18 and 15, respectively).

AIS

AIS, containing IgG1 and IgG2 antibodies, were obtained as previously described [22] by cross-hyperimmunization with spleen cell membranes of S.2 and S.13 for class II antigens (anti-Ia 1, 3, 7 and anti-Ia 2, 4) and by cross-hyperimmunization of BIO-AD and S.13 for class I antigens (anti-B1, S1 and anti-B3). The high specificity of these AIS was previously demonstrated by *in vivo* and *in vitro* tests [20].

These 4 AIS were studied for each guinea pig strain; thus according to the corresponding target they were either specific (anti-B1 S1 on S.2 and S.13, anti-B3 on BIO-AD, anti-Ia 2, 4 on S.2, anti-Ia 1, 3, 7 on S.13 and BIO-AD) or non-specific with reverse combinations. As results were homogeneous and reproducible we could pool them in order to discriminate between specific or non-specific class I or class II AIS.

Estrogen treatment

Guinea pigs were treated with estradiol benzoate (Roussel, France): 1 mg was dissolved in 0.2 ml of sterile arachis oil and injected s.c. into each animal once a week. Blood was collected 7 days after the last injection of a course of three.

Cell preparations

PBL were obtained from the retro-orbital sinus blood or by cardiac puncture and collected on 10% EDTA (1:2 v/v blood). After sedimentation with 3% Dextran (T500-Pharmacia) at 1 g for 30 min, PBL were washed 3 times with phosphate buffered saline (PBS), 0.02 M sodium azide (NaN3) and adjusted to 2.5×10^6 cells/ml in PBS, NaN3 supplemented with 0.5% bovine serum albumin (BSA).

The cellular composition was determined after staining with May–Grünwald–Giemsa stain; 43% PMN, 53% MN and 4% Kurloff cells [23]. After estradiol treatment the percentage of Kurloff cells reached 13% and the MN percentage decreased to 43%.

SPA-SRBC

All reagents were prepared in 0.15 M NaCl. 50 μl of SPA (2 mg/ml) and 1 ml of 3 × washed SRBC (6.25%) were mixed with the appropriate amount of 0.1% chromium chloride, pH 5, for 5 min. Conjugation was stopped by adding 9 ml of PBS. After several washings, a 1% SPA-SRBC suspension was prepared in PBS–BSA–NaN3 for use in further steps. According to our previous experience, we only used SPA-SRBC preparations giving at least 90% of rosetting cells with a 1/10,000 dilution of anti-class I serum and appropriate target cells.

SPA-SRBC rosette assay

PBL GPL-A antigens were identified by the SPA rosetting method [22] first described by Ghetie *et al.* and modified by Sandrin *et al.* [24]. Briefly 25 μl of PBL were incubated for 30 min at 4°C with appropriate dilutions of the 4 AIS. After 3 washings with PBS–NaN3, cells were suspended in 25 μl of a PBS–BSA–NaN3 mixture. They were mixed with 25 μl of 1% SPA-SRBC in PBS–BSA–NaN3 and centrifuged for 5 min at 200 *g* to promote rosette formation. The next day, PBL were stained with methylviolet and the percentage of rosetting cells (cells binding at least 3 SPA-SRBC) was scored in a hematocytometer. The counts constituted the results for whole fresh PBL suspensions.

Identification of the rosetting cells

The pattern of the rosetting cells: Kurloff, MN and PMN was determined from cytocentrifuged cellular suspensions stained with May–Grünwald–Giemsa or by color rapid reagent (Scientec, France). MN corresponded to the total MN minus Kurloff cells. The

percentage of rosetting cells of each type was established after enumerating all the cells present on the smear, and the number of bound SPA-SRBC on 100 cells was determined for each cell type (a total of 2445 rosetted MN, 2633 rosetted Kurloff cells and 2374 rosetted PMN were examined in normal, and estrogen-treated animals). Complete rosettes were leukocytes completely surrounded by coated SPA-SRBC and incomplete rosettes were cells only partially surrounded by SPA-SRBC.

Statistics

Obtained data were statistically analyzed by comparison of fresh rosetting PBL percentages and by comparison of the means of SPA-SRBC rosetted per 100 cells, with Student's *t*-test.

RESULTS

Similar results were obtained for all guinea pig strains used.

Whole fresh PBL suspensions

Figure 1 shows the results obtained with whole fresh PBL suspensions. The percentages of rosetting PBL for class I antigens were about 80–90% for normal guinea pig cells incubated with all dilutions of anti-class I specific AIS, ranging from 1/10 to 1/40,000. For estrogenized guinea pigs these percentages decreased and we observed a zone effect: only 65% of PBL formed rosettes at the 1/10 dilution, 75% with the 1/100–1/20,000 dilutions and 68% with the 1/40,000 dilution (Fig. 1A); these decreasing percentages were statistically significant in comparison with the normal group ($P < 0.01$ and $P < 0.05$). Moreover, rosettes from normal guinea pigs were complete whereas those from the estrogenized group were incomplete. With class II specific AIS diluted at 1/5 and 1/20, only a small percentage of PBL were Ia positive: 30% in the normal group and 15% in the estrogenized group ($P < 0.01$) (Fig. 1B).

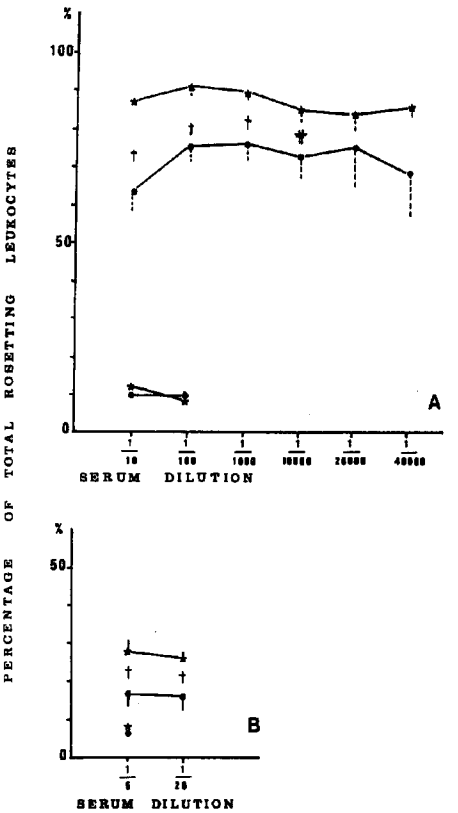

Fig. 1. Class I (A) and class II (B) MHC antigens expressed as a percentage of rosetting leukocytes. Whole fresh preparations of PBL. Normal (•) and estrogenized (•) guinea pigs (18 and 15 animals, respectively). Results are expressed as mean ± SD (†$P < 0.01$, ‡$P < 0.05$). Bottom: non-specific reaction with reverse combinations of AIS.

Smears

Table 1 shows the percentage of Kurloff, MN and PMN rosetted cells established after enumerating all the cells present on a smear.

Table 1. Percentage of rosetting Kurloff, MN and PMN cells, determined from enumerating rosettes on cytocentrifuged smears

Alloimmune sera	Specific class I (1/100)	Specific class I (1/40,000)	Non-specific class I (1/100)	Specific class II (1/5)	Non-specific class II (1/5)	Cell alone (control)
Kurloff cells						
Normal	93	100	0	38.7	0	0
Estrogenized	73.3	72	5.4	17	0	2
MN						
Normal	87.9	88	14.4	73.8	16	22.5
Estrogenized	44.8	34.2	13.1	42.5	0	5.4
PMN						
Normal	91	78.5	6	0	0	0
Estrogenized	87	74.7	4.8	0	0	0

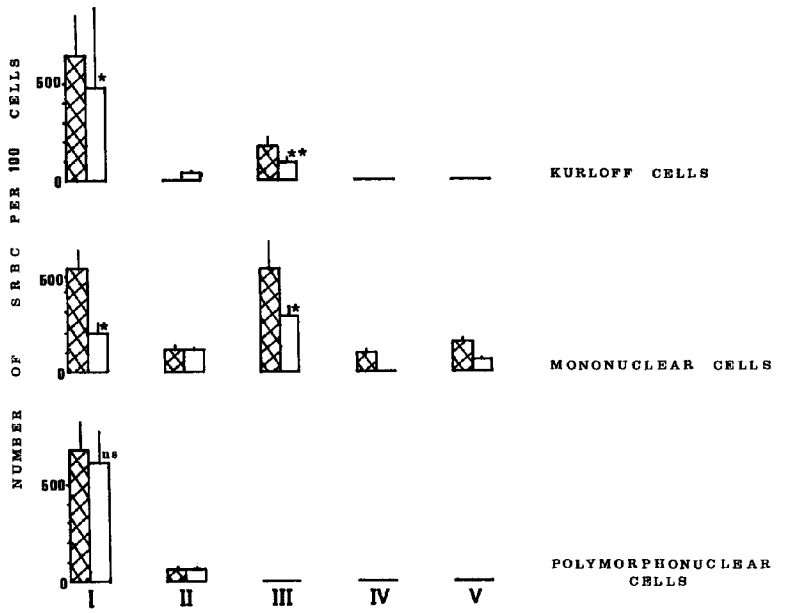

Fig. 2. Number of bound SPA-SRBC to 100 cells, incubated with specific (I) or non-specific (II) 1/100 diluted anti-class I AIS, with specific (III) or non-specific (IV) 1/5 diluted anti-class II AIS, or without serum (V). Normal ⊠ or estrogenized □ guinea pigs. (Bars = SD). Significance of estrogenized data vs normal data: $*P < 0.001$, $**P < 0.05$, ns = non-significant.

Comparatively Fig. 2 shows the number of bound SPA-SRBC per 100 cells of each cell type after enumeration of every SPA-SRBC constitutive of each rosette. These values were established for samples incubated either with the different AIS or without serum.

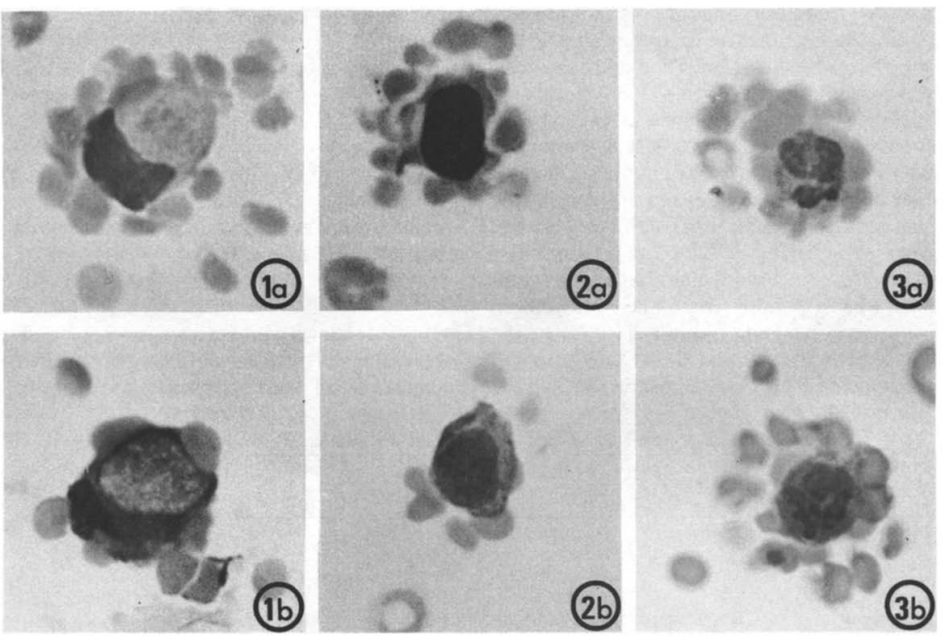

Fig. 3. SPA-SRBC rosetted PBL incubated with 1/100 diluted anti-class I AIS. Kurloff (1), MN (2) and PMN (3) rosetted cells in normal (a) and estrogenized (b) guinea pigs (×1500).

Kurloff and PMN cells from normal and estrogenized groups did not spontaneously bind SPA-SRBC though some MN cells did.

With class I-specific AIS, the percentage of rosetting Kurloff MN and PMN cells was between 85 and 100% in normal guinea pigs.

In the estrogen-treated group, a marked decrease of 30 and 50% in the number of rosetting cells was scored for Kurloff and MN cells, respectively. There was no significative difference for PMN in any group.

With class II-specific AIS, the rosetting Kurloff and MN cells was about 50% lower in the estrogenized group than in the normal group. PMN did not bind class II-specific AIS.

For the normal group, almost all rosettes were complete (i.e. SRBC bound all around the cell, even on more than one row); in contrast, in the estrogenized group, rosettes were incomplete as cells were only partially surrounded by SPA-SRBC (Fig. 3).

DISCUSSION

The main finding of this study was that estradiol modifies the expression of GPL-A antigens on PBL, but not on PMN cells.

It was possible to obtain these results as the rosetting method is highly sensitive [20] provided that the conjugation of SPA is well controlled (see Materials and Methods section). This method was recently used to detect T-cell activation antigen (Tac) positive cells in the tonsillar T-cell fraction as conventional immunofluorescence assays were not sufficiently sensitive to detect IL2 receptors on lymphocytes activated *in vivo* [25]. The rosetting and flow cytofluorometry methods were compared for the expression of Tac and Ia antigen, on circulating human T-cells [26] and of 25 cell surface alloantigens on mouse lymphocytes and neutrophils [27]. There was no discrepancy between the two techniques except for mouse Ly 25-1 + lymphocytes which were only detected by the rosetting method: the disparity between rosetting (80%) and cytofluorometric (12%) techniques was interpreted to be due to the very low Ly 25-1 density on lymphocytes. By using the rosetting method and by counting the number of bound SPA-SRBC per 100 cells, we could approximately evaluate the density of cell surface GPL-A antigens: in previous experiments (data not shown) we followed the expression of GPL-A antigens on spleen cells during solubilization with octyl-β-D glucopyranoside, a non-cytolytic detergent and we observed a correlation between the increase in the amount of solubilized antigens in the incubation medium (immunodot blotting procedure) and the decrease in the number of bound SPA-SRBC on splenic cells (rosetting method); complete rosettes became incomplete and then gradually disappeared. These independent measures suggested that the number of bound SPA-SRBC provides a quantitative index of the expression of cell surface antigens.

As expected, class I antigens were better represented on normal PBL than class II antigens: almost all cells were positive for class I GPL-A antigens up to the 1/40,000 dilution of AIS, whereas a 1/5 dilution of class II-specific AIS gave only 30% positive cells with a fresh suspension. On whole fresh cell suspensions, the same anti-class II-specific AIS diluted to 1/20 could detect GPL-A antigens on 80% of L2C-N leukemia cells and 80% of lymph node cells obtained from guinea pigs treated with complete Freund's adjuvant 3 weeks before.

The percentage of rosetting cells was 30% for whole fresh cell suspensions and 75% for MN cytocentrifuged cells as PMN were class II negative. Kurloff and PMN cells had no surface IgG, as demonstrated by the lack of spontaneous binding of SPA-SRBC to cells incubated alone without serum. We also found that Kurloff cells are IgM negative as no antibody binding was observed either by the rosetting method or a peroxidase–antiperoxidase immunostaining technique, after incubation with rabbit anti-guinea pig IgM (unpublished results).

Incubation of PBL from normal and estrogenized guinea pigs with AIS directed against GPL-A antigens not borne by the target cells (non-specific anti-GPL-A AIS) did not induce any rosetting cells. This demonstrated that, under our experimental conditions, we could not detect any Fc receptors for IgG on these cells.

In this paper we described the modulation of MHC antigen expression by supraphysiological doses of estradiol on different families of guinea pig blood leukocytes. MHC antigens present on PMN, which are not involved in immunity, showed no variation of their expression; the expression of those from MN, including Kurloff cells, which are involved in immunity or in natural killer effect, decreased after experimental estrogen treatment: indeed supraphysiologi-

cal doses of estradiol are known to decrease the cell mediated immunity responses [28, 29].

As far as the mechanism of this modulation is concerned, the first hypothesis is a binding of estrogen with specific receptors of lymphoid cells and then a modification of DNA transcription and of antigen synthesis. However, estradiol receptors are present mainly on reticulo-epithelial components of lymphoid organs and not on lymphoid cells [30]; as an exception, estradiol receptors are characterized only on OKT8-positive cells in man [31]. In the guinea pig Kurloff cells, only the presence of some low affinity estrogen binding sites of type II was detected, but the typical type I estrogen receptor is actually absent [32]; therefore the binding of steroid hormones with cell receptors is not possible for all the lymphoid cells. But the fact that Kurloff cells do not possess any typical type I estrogen receptor is not incompatible with estrogenic modulation of these cells. Many authors showed that gonadal steroid could act through a non-genomic mechanism by directly binding to specific membrane receptors [33]. Moreover, we cannot exclude any indirect effect of estradiol on lymphoid cells since our experiments were done *in vivo* and not directly *in vitro*.

The medical interest of this modulation is obvious. In rodents and in man it is well known [21] that hormonal immunity, cell mediated immunity, allergy, autoimmunity and natural killer activity are more or less marked in males and in females, and for the females according to the estrous cycle (e.g. high immunoglobulin levels in infectious diseases, allergic predispositions and systemic lupus erythematosus, are more frequent in women, and so on). Although this paper is only devoted to estrogen action, it shows a direct influence of the hormonal impregnation on cells interfering with immunity or natural killer activity.

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