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Effect of uterine immunization and oestradiol on specific IgA and IgG antibodies in uterine, vaginal and salivary secretions

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SUMMARY

Levels of IgA and IgG antibodies were measured in uterine and vaginal secretions to examine the effect of uterine immunization on the genital tract humoral immune system. When ovariectomized animals were immunized on Day 0 and boosted 13 days later by placing sheep erythrocytes (SRBC) directly in the uterine lumen (UT/UT) immunization), a pronounced IgA and IgG antibody response was detected in uterine secretions measured on Day 26. This response was 20–30-fold greater than that measured following Peyer's patch immunization and boosting (PP/PP) and Peyer's patch immunization followed by uterine boosting (PP/UT). In contrast to uterine antibody responses that were oestradiol-dependent following PP/PP and PP/UT immunization, UT/UT immunization resulted in IgA and IgG antibody responses that were hormonally independent. To determine whether immunological information is distributed beyond the immediate site of immunization, ovariectomized rats were immunized and boosted by injection of SRBC into one uterine horn. When uterine secretions from the contralateral (non-immune) horns were analysed, IgA and IgG antibodies were found in uterine secretions after oestradiol stimulation. IgA and IgG antibodies were also present in vaginal secretions following UT/UT immunization and ligation of uteri at the utero–cervical junction. This response was hormonally dependent in that vaginal antibody levels were lowered by oestradiol treatment. IgG but not IgA antibodies were also found in saliva of UT/UT immunized animals. Oestradiol had no effect on salivary IgG levels in contrast to those of the genital tract. In summary, these experiments indicate that immunization of uteri can elicit pronounced IgA and IgG antibody responses in uterine secretions and this response is not altered by oestradiol. Moreover, immunization at one site in the genital tract results in the appearance of antibodies at other uterine sites (the contralateral-non-immunized horn), in vaginal secretions, in serum and at other mucosal sites, such as the salivary glands.

INTRODUCTION

The female genital tract is a part of the mucosal immune system which confers protection against both bacterial and viral pathogens (Vaerman & Ferin, 1974; Ogra, Yamanaka & Losonsky, 1981). Depending on the site examined in the genital tract, effector mechanisms of immunity may be either plentiful or sparse. In the uterus, some studies have demonstrated the presence of immunocompetent cells, while others have reported very few, if any, IgA- and/or IgG-producing lymphocytes (Lippes *et al.*, 1970; Rebello, Green & Fox, 1975; Kelly & Fox, 1979). The cervix and vagina appear to be richly endowed with both IgA and IgG lymphocytes (Rebello *et al.*, 1975; Tourville *et al.*, 1970). One explanation for these differences is that a resident population of microbial antigens is present in the cervix and vagina but not in the uterus (Vaerman & Ferin, 1974; Larsen, Markovetz & Galask, 1976; Wira & Merritt, 1977). When antigens enter the uterus, their presence is transient and correlates with copulatory activity (Parr & Parr, 1985a).

The female sex hormones have marked effects on the mucosal immune system in the female reproductive tract (Wira & Sandoe, 1977). Previously, our studies have shown that levels of IgA and IgG and secretory component change in the uterus and vagina during the reproductive cycle and following hormone treatment (Wira & Sandoe, 1980; Sullivan, Underdown & Wira, 1983; Wira & Sullivan, 1985). We and others have found that oestradiol regulates the mucosal immune system by (i) controlling IgA and IgG movement from blood to tissue (Sullivan & Wira, 1983, 1984); (ii) stimulating the movement of IgA-positive lymphoid cells into the female genital tract (Wira *et al.*, 1980; McDermott, Clark & Bienenstock, 1980; Rachman *et al.*, 1983; Parr & Parr, 1985b); and (iii) regulating the transfer of immunoglobulins from tissue to lumen (Sullivan & Wira, 1983; Wira, Sullivan & Sandoe, 1983). In the latter case, IgA is transported through uterine epithelial cells prior to release into

Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; PP, Peyer's patch; RIA, radioimmunoassay; SRBC, sheep erythrocytes; UT, uterus.

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uterine secretions bound to secretory component that is stimulated by oestradiol (Sullivan *et al.*, 1983; Wira *et al.*, 1983). IgG also moves through uterine cells but occurs via a mechanism different from that which controls IgA transfer (Templeman & Wira, 1987).

More recently, we have found that Peyer's patch (PP) and intraperitoneal immunization of intact female rats with sheep red blood cells (SRBC) leads to the appearance of specific IgA and IgG antibodies in uterine and vaginal secretions (Wira & Sandoe, 1987). When analysed in ovariectomized animals, PP immunization resulted in uterine antibody accumulation only after animals were treated with oestradiol prior to killing. In contrast, vaginal anti-SRBC antibodies were present in uterine-ligated rats and were reduced when animals were treated with oestradiol.

In light of the need to identify optimal endocrine conditions and effective routes of immunization to induce genital tract protection against venereal diseases, including AIDS (Peterman & Curran, 1986), the present study was undertaken (i) to examine the effect of uterine immunization on the magnitude of the antibody response to SRBC relative to that obtained following PP immunization; (ii) to determine whether the uterine response obtained following uterine immunization is dependent on oestradiol; and (iii) to evaluate whether immunological information is shared between various sites within and distal to the reproductive tract when a discrete site within the uterus is exposed to antigen.

MATERIALS AND METHODS

Animal and surgical procedures

Adult female Sprague–Dawley rats (200–300 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA). Animals were maintained in a constant-temperature room with fixed light/dark intervals of 12 h and allowed food and water *ad libidum*. Animals were ovariectomized 7–10 days before the start of each experiment.

Immunization procedures

For immunization studies, sheep red blood cells (SRBC) were purchased from Gibco Laboratories (Grand Island, NY). Just prior to use, cells were washed five times in saline (0·15 M) and centrifuged (1200 *g* for 2 min) to obtain packed cells (1×10^{10} cells/ml). To immunize animals via the Peyer's patches (PP), rats were ether-anaesthetized and intestines were exteriorized through an abdominal midline incision. Packed SRBC (100 μl) were injected directly into Peyer's patches (5–10 μl/PP; 15–20 PP/rat) with a 26-gauge needle and syringe (TB). This approach has been shown previously by Andrew & Hall (1982) to elicit antibody responses comparable to those seen in bile and sera following oral immunization, while having less within-group variation. Packed cells can be injected because erythrocytes are semi-rigid and therefore retain some fluid between cells after centrifugation. For intrauterine immunization, uterine horns were exteriorized through a midline incision and injected with SRBC at the utero–cervical junction. Immediately following deposition of SRBC (20–30 μl of packed cells) into the uterine lumen, each uterine horn was ligated at the utero–cervical junction prior to 26-gauge needle removal by tying with cotton-covered polyester thread. Non-immunized horns were also ligated at this time. Animals were boosted by depositing SRBC into the uterine lumen at the oviductal end of each uterine horn prior to ligation to prevent leakage of luminal contents into the peritoneal cavity.

General procedures

Uterine secretions were obtained from rats, as described previously (Wira & Sandoe, 1987). Briefly, animals were anaesthetized with sodium pentobarbitol (60 mg/kg) and, following perfusion of the uterine vasculature with saline, uterine contents were collected by luminal perfusion with saline (0·2 ml). Vaginal fluids were collected by flushing vaginae with 200 μl of saline prior to the start of hormone treatment. Blood was collected by direct cardiac puncture and allowed to clot for 1 hr at room temperature. Clots were detached from the tube wall by rotating a wooden rod around the perimeter of each tube (ringing) and maintained at 3° for an additional 24 hr to allow further clot retraction. Uterine and vaginal secretions and blood were centrifuged (12,000 *g* for 4 min) and frozen at −20° until assayed. Saliva was collected for 5 min from anesthetized rats by injecting pilocarpine HCl (1 mg/100 g, subcutaneously).

Measurement of specific antibodies

Specific IgA and IgG antibodies directed against SRBC were measured by radioimmunoassay, as described previously (Wira & Sandoe, 1987). Briefly, purified mouse monoclonal anti-rat IgA (Sullivan *et al.*, 1986) and heavy-chain specific goat anti-rat IgG (Miles Laboratories, Elkhart, IN), which was further purified by protein A chromatography, were iodinated by the Iodo-Gen method (Markwell & Fox, 1978). To run each assay, uterine and vaginal secretions and sera were diluted in Dulbecco's buffer (PBS/BSA; 0·15 M NaCl, 2·7 mM KCl, 1·5 mM KH_2PO_4, 6·5 mM Na_2HPO_4, pH 7·1) containing 1·0 mg/ml bovine plasma albumin (Calbiochem, La Jolla, CA). SRBC (100 μl; 1×10^9 cells) were added to secretions and standards (50 μl) and suspensions were incubated with vibration for 1 hr. Following washing with PBS/BSA (1·0 ml) and centrifugation (3000 *g* for 6 seconds), cells were resuspended by vortexing in 100 μl of ^{125}I-labelled mouse monoclonal anti-rat IgA or ^{125}I-labelled goat anti-rat IgG and incubated for an additional 2 hr. SRBC in the IgA assay were washed with PBS/BSA (1·0 ml) while IgG assay cells were washed in saline containing Tween 20 (0·05%). Following centrifugation (12,000 *g* for 2 min), pellets were counted in a Multiprias autogamma counter (Packard Company, Downers Grove, IL). Reproducibility of these assays has been reported previously (Wira & Sandoe, 1987). Statistical significance of differences between groups of data were calculated by Student's *t*-test.

Steroid preparations

Oestradiol, progesterone, and dihydrotestosterone were purchased from Calbiochem (La Jolla, CA). Dexamethasone was acquired from Steraloids (Pawling, NY). Oestradiol was solubilized in absolute ethanol, evaporated, and resuspended in saline. Control animals received saline containing an equivalent volume of alcohol residue. Progesterone, dihydrotestosterone and dexamethasone were suspended in saline by glass–glass homogenization. All injections (0·1 ml) were given subcutaneously on a daily basis, as described in the Results.

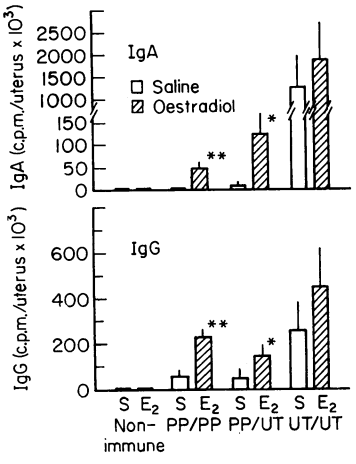

Figure 1. Influence of oestradiol and route of immunization on the presence of anti-SRBC-specific IgA and IgG antibodies in uterine secretions. Ovariectomized animals were immunized with SRBC injected into Peyer's patches (PP) or instilled intraluminally into one uterine horn (UT). Animals were immunized (primary, Day 0) via the Peyer's patches and boosted (secondary, Day 13) either via the Peyer's patches (PP/PP), or the uterus (PP/UT) or were immunized and boosted by placing SRBC directly into the uterine lumen (UT/UT). Non-immunized animals were sham-operated at the time of primary and secondary immunization. Animals were injected with 0·1 ml saline (S) or oestradiol (E_2; 1·0 μg/day) for 3 days prior to killing 24 hr after the last injection on Day 26 post-primary immunization. Bars represent the mean ± SE values of samples taken from five to six animals/group. *Significantly ($P < 0.05$) greater than saline-treated immunized controls, **significantly ($P < 0.01$) greater than saline-treated immunized controls.

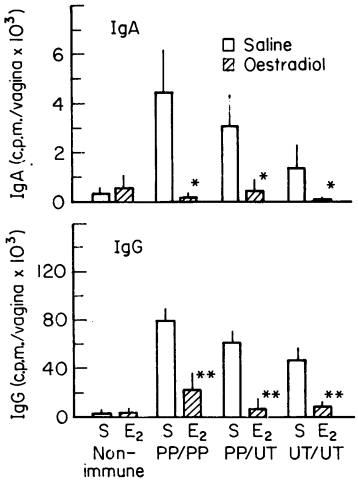

Figure 2. Effect of oestradiol and immunization route on specific IgA and IgG antibodies in vaginal secretions of ovariectomized rats. Animals were immunized and boosted via Peyer's patches (PP) or uterus (UT) as described in detail in Fig. 1. Bars represent mean ± SE of each group. *Significantly ($P < 0.05$) lower than saline-treated immunized animals; **significantly ($P < 0.01$) lower than saline-treated immunized controls.

RESULTS

Comparison of routes of immunization and oestradiol treatment on specific antibodies in uterine and vaginal secretions

The effects of uterine (UT) and Peyer's patch (PP) immunization and hormone treatment on anti-SRBC IgA and IgG antibodies in the uterine flushings from ovariectomized rats are shown in Fig. 1. When animals received primary and secondary immunizations of SRBC directly into the uterine lumen (UT/UT), anti-SRBC IgA and IgG antibodies were found in uterine secretions. This response was hormonally independent because daily administration of oestradiol for 3 days prior to uterine fluid collection had no effect on IgA or IgG anti-SRBC antibody levels. As a part of this study, uterine antibody levels following UT/UT immunization were compared to those obtained after Peyer's patch immunization followed by either Peyer's patch (PP/PP) or uterine (PP/UT) boost. As seen in Fig. 1, irrespective of hormone treatment, UT/UT immunization resulted in IgA and IgG antibody responses that were 20–30-fold and 2–5-greater, respectively, than those obtained following PP/PP or PP/UT immunization. In contrast to UT/UT immunization, oestradiol treatment of PP/PP- and PP/UT-immunized animals was required for IgA and IgG antibodies to accumulate in uterine secretions.

Vaginal antibody levels were also measured and are shown in Fig. 2. When animals were UT/UT immunized, vaginal IgA and IgG antibody levels of saline-treated controls were significantly higher than those measured in the vaginal secretions of non-immunized animals. When animals were treated with oestradiol, IgA- and IgG-antibody levels were reduced relative to saline-treated immunized animals. Similar vaginal changes were observed with PP/PP- and PP/UT-immunized animals, in that oestradiol lowered antibody levels either towards or to background values measured in non-immunized animals.

Influence of hormones on anti-SRBC antibodies in uterine secretions

The effect of various hormones on the accumulation of specific IgA antibodies in the uteri of ovariectomized rats is shown in Fig. 3. When animals were PP/UT immunized and treated with

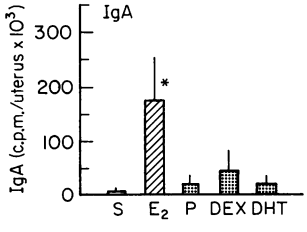

Figure 3. Influence of various steroids on specific IgA-antibody levels in uterine secretions of SRBC-immunized animals. Ovariectomized rats were immunized via the Peyer's patches and boosted 13 days later by instillation of SRBC into one uterine horn. Animals received three daily injections (0·1 ml) of oestradiol (E_2; 1·0 μg/day), progesterone (P; 2·0 mg/day), dexamethasone (DEX; 2 mg/day), dihydrotestosterone (DHT; 2·0 mg/day) or saline (S) and were killed 24 h after the third injection on Day 26 post-primary immunization. Each bar represents the mean ± SE of four animals/group.*Significantly ($P < 0.05$) greater than saline-treated immunized animals.

Table 1. Concentration of anti-SRBC IgA and IgG antibodies in serum and saliva following UT/UT immunization and treatment of ovariectomized rats with oestradiol

		Non-immunized		UT/UT-immunized	
Source	Antibody	Saline	Oestradiol	Saline	Oestradiol
Serum	IgA (c.p.m./ml × 10^3)	9±6·1	0±0	25±14	21±7·9*
	IgG (c.p.m./ml × 10^6)	21±1·3	17±4	378±44*	480±40*†
Saliva	IgA (c.p.m./ml × 10^3)	0	0	0	0
	IgG (c.p.m./ml × 10^3)	0	0	65±28*	72±11*

Animals were immunized and treated with oestradiol (1 μg/day) or saline for 3 days prior to killing. Each value represents the mean ± SE of five to six animals/group.

*Significantly ($P < 0.01$) greater than non-immunized animals.

†Significantly ($P < 0.001$) greater than saline UT/UT-immunized animals.

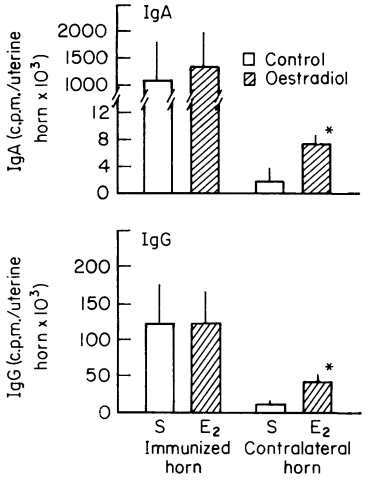

Figure 4. Effect of immunization and boost in one uterine horn on the presence of antibodies in the non-immunized contralateral uterine horn. Ovariectomized rats received primary immunization (Day 0) by instillation of SRBC (20–30 μl/horn) into one uterine horn. Following injection of SRBC into the lumen, uteri were ligated at the utero–cervical junction to prevent leakage. On the day of boost (Day 13), SRBC were injected into the oviductal end of the immunized horn, which was then ligated. Animals were treated with 0·1 ml of oestradiol (E_2, 1 μg/day) or saline (S) for 3 days prior to killing, 24 hr after the last injection (Day 26 post-primary immunization). Bars represent the mean ± SE of four animals/group. *Significantly ($P < 0.05$) greater than immunized saline controls.

oestradiol for 3 days prior to killing IgA antibody levels were significantly higher than those present in saline-immunized controls. In contrast, progesterone, dihydrotestosterone and dexamethasone, a synthetic glucocorticoid, had no effect on the accumulation of antibodies in uterine secretions.

Effect of uterine immunization on the presence of antibodies in non-immunized uterine horn secretions, serum and saliva

To establish whether immunological information is shared between individual horns of the bicornuate uterus, both uterine horns of UT/UT-immunized animals were ligated at the utero–cervical junction at the time of immunization to prevent transfer of uterine fluid and antigen from one horn to the other. Animals were treated with saline or oestradiol for 3 days prior to killing on Day 13 post-boost. As seen in Fig. 4, significant amounts of IgA and IgG antibodies accumulated in the immunized uterine horns of saline- and oestradiol-treated animals. This response, as described above, was hormonally independent. In contrast, when measured in the non-immunized (contralateral) horns, IgA and IgG antibodies were present and found to be hormonally dependent. Anti-SRBC antibody levels were significantly higher in uterine secretions of oestradiol-treated animals than in secretions from saline-injected animals. This finding indicates that the antibody response to UT/UT immunization, while hormonally independent in immunized horns, is oestradiol dependent in non-immunized uterine horns.

To determine whether immunological information is shared with mucosal surfaces distal to the female genital tract, antibody levels were measured in sera and saliva of UT/UT-immunized rats. As seen in Table 1, relative to non-immunized controls, uterine immunization resulted in significant increases in anti-SRBC IgG antibodies in both serum and saliva. When oestradiol was administered daily for 3 days prior to sample collection, levels of IgG antibody in serum were significantly higher than those measured in immunized animals that received saline. In contrast, oestradiol had no effect on the levels of IgG antibodies in saliva of immunized rats. A comparison of IgG antibody levels in saliva and serum from these animals indicates that saliva had significantly lower IgG antibody levels than did serum. Nevertheless, salivary levels measured were sufficient to agglutinate SRBC in individual assay tubes.

As a part of these studies, serum and saliva were also analysed for anti-SRBC IgA antibodies. As shown in Table 1, IgA antibodies against SRBC were present in sera of immunized oestradiol-treated rats but were not consistently found in either sera (saline-treated) or saliva of immunized animals. In other experiments (not shown), low levels of IgA antibodies have been periodically measured in serum (saline treated) and in saliva following UT/UT immunization. The lack of a consistent

response may reflect either the rapid clearance of IgA from serum by hepatocytes or the migration of limited numbers of IgA-producing lymphocytes to the salivary glands.

DISCUSSION

The present studies demonstrate that specific antibodies against SRBC can be measured in uterine and vaginal secretions following local intrauterine stimulation by antigen. Our findings also indicate that uterine immunization and uterine boost (UT/UT) stimulate IgA and IgG antibody responses in uterine secretions that are hormonally independent and greater than those measured following PP/PP or PP/UT immunization. Uterine (UT/UT) immunization also resulted in the presence of specific antibodies in nonimmunized (contralateral) uterine horns, in vaginal secretions, sera and saliva. Contralateral uterine and the vaginal responses, as well as serum antibody levels, were found to be dependent on oestradiol.

Previously, we reported that oral (feeding) and intraperitoneal immunization of intact female rats with SRBC results in the accumulation of both IgA and IgG antibodies in uterine and vaginal secretions (Wira & Sandoe, 1987). When SRBC were placed directly into Peyer's patches to reduce biological variation of the oral response (Andrew & Hall, 1982), specific antibodies in uterine and vaginal secretions were shown to be dependent on oestradiol; antibody accumulation in uterine secretions increased in response to oestradiol at a time when it was inhibited in cervico–vaginal secretions (Wira & Sandoe, 1987). The present studies extend these observations by demonstrating that primary Peyer's patch immunization followed by uterine boosting fails to significantly increase uterine antibody levels beyond those measured after PP/PP immunization. More importantly, the present study demonstrates that intrauterine immunization with non-replicating antigen results in an antibody response that is more pronounced than that seen following oral, Peyer's patch, or Peyer's patch followed by uterine boosting. Replicating antigens, such as Brucella abortus and Vibrio fetus, elicited uterine antibody titres that were higher than those measured in serum (Kerr, 1955; Corbeil *et al.*, 1974). In contrast, when non-replicating antigens, such as diphtheria toxoid or horseradish peroxidase, were placed in uteri, little or no IgA responses were measured (Bell & Wolf, 1967; McAnulty & Morton, 1978). Uterine immunization in women was also investigated by Ogra & Ogra (1973). When inactivated polio virus was placed within the uterine lumen, women developed titres of IgG class antibodies in uterine secretions. In contrast, Vaerman & Ferin (1974) found no antibody production in women following intrauterine immunization with horseradish peroxidase. Our findings of pronounced uterine IgA and IgG antibody responses with SRBC indicate that the local immune system in the uterus has the potential for immune responsiveness to non-replicating antigen (SRBC). One explanation for the differences observed may be that, in our study, antigen was retained in the uterus by ligation of the uterine horn. It may be that a critical time interval of exposure to antigen is essential for immune responsiveness. Alternatively, since our animals were ovariectomized, their endocrine balance at the time of immunization was different from that studied previously. Experiments are presently under way in our laboratory to examine both possibilities.

The presence of antibodies in uterine secretions following intrauterine immunization may reflect either local synthesis or serum transudation of antibodies into the uterine lumen. Lymphoid cells are known to enter mucosal tissues, such as the intestine, etc., in response to antigen exposure within Peyer's patches (Bienenstock & Befus, 1980). Whether a comparable infiltration of immunocomponent B and T lymphocytes enters the uterus in response to antigen exposure, remains to be determined. Since Ia^+ cells, which function as accessory cells for T-cell activation *in vitro*, are present in the uterus (Head, Miller & Kresge, 1986), the possibility exists that lymphocytes, in response to antigen, enter the uterus and synthesize antibodies locally. Alternatively, since lymphatics, though small and sparse, do exist in the virgin uterus (Head & Seelig, 1984), their presence suggests that antigens in the uterine lumen may have access to draining lymph nodes. Following antigen presentation, B lymphocytes may migrate to distal sites as well as to the uterus. If antigen is distributed beyond the confines of the genital tract, then in addition to local antibody production at mucosal surfaces, significant amounts of immunoglobulins may be transmitted from serum during mucosal inflammation (Hanson & Brandtzaeg, 1973). While the origin of uterine antibodies remains to be established, it appears unlikely that the uterine response is due to serum transudation. IgA/IgG antibody ratios in serum were found to be < 1 while those measured in uterine secretions were > 1. It therefore seems unlikely that inflammation with resulting serum transudation is responsible for the presence of antibodies in the immunized uterine horns.

An unexpected finding in this study was that the movement of antibodies into uterine secretions following UT/UT immunization was independent of oestradiol stimulation. Previously, we have shown that IgA movement into genital tract secretions is controlled, in part, by oestradiol regulation of secretory component, which is thought to be the receptor for IgA (Sullivan *et al.*, 1983; Wira & Sullivan, 1985). One explanation for the absence of an oestradiol effect on IgA antibody levels is that under the conditions of antigenic stimulation, the movement of IgA is limited by receptor availability. Alternatively, if receptors are not saturated then synthesis of IgA may be rate limiting.

Local antibody production following vaginal or cervical deposition of antigen indicates that the lower genital tract (cervix and vagina) is immunologically responsive (Kerr, 1955; Bell & Wolf, 1967; Yang & Schumacher, 1979; Parr & Parr, 1988) and that this response is influenced by oestradiol (Rank *et al.*, 1982). Our studies extend these observations by demonstrating that immunological information is shared between the uterus and vagina when both compartments are separated by uterine ligation. Previously, we and others have found that gastrointestinal immunization results in antibody accumulation in the genital tract (Ogra & Ogra, 1973; Stuern & Schneweis, 1978; Allardyce, 1984; Wira & Sandoe, 1987). The present study demonstrates that the uterus, in addition to being a recipient of immunological information, also functions as a site from which immunological information is disseminated to other sites in the body. In addition to measuring anti-SRBC antibodies in the vaginae of uterine-immunized animals, we found levels of IgA and IgG antibodies in the secretions of the non-immunized (contralateral) horn following oestradiol stimulation that were comparable to those measured in saliva and in vaginal secretions of saline-treated animals. At present, the mechanisms involved in the appearance of antibodies at distal sites is

unknown. It appears likely, however, that IgG antibodies in the contralateral horn may be of serum origin. Previously, we found that IgG, in response to oestradiol, moved down a concentration gradient from blood to tissue to lumen and that intravenously injected radiolabelled IgG accumulated in uterine secretions of oestradiol-treated animals (Sullivan & Wira, 1983, 1984). A similar transfer of specific IgG antibodies from serum to the contralateral horn and into saliva may occur following UT/UT immunization. IgA antibodies in the contralateral horn are less likely to be of serum origin in light of our earlier finding that elevated levels of serum polymeric IgA did not result in elevated uterine levels of IgA following oestradiol stimulation (Wira & Stern, 1986). Whether lymphocytes enter the genital tract in response to antigens, as they appear to during the oestrous cycle, and in response to oestradiol (McDermott *et al.*, 1980; Wira *et al.*, 1980; Rachman *et al.*, 1983; Parr & Parr, 1985), and then migrate to the non-immunized uterine horn and vagina where they synthesize IgA antibodies prior to transport by secretory component remains to be established.

Previously, we found that serum immunoglobulin G levels are elevated at the proestrous stage of the reproductive cycle (Wira & Sandoe, 1980). Others have shown that systemic (serum) immunity can develop from vaginal (Yang & Schumacher, 1979) and intrauterine (Lande, 1986) antigen exposure and that systemic (subcutaneous) immunization results in serum antibody levels that are enhanced when animals are treated with oestradiol (Trawick & Bahr, 1986). Our findings indicate that oestradiol treatment, relative to saline treatment of immunized animals, also generates higher anti-SRBC IgG antibody levels in serum following uterine immunization. Whether this is due to direct hormone effects on B-cell antibody synthesis as has been reported previously (Meyers & Peterson, 1985), remains to be established.

The immunological implications of multiple sites available for immunization to confer genital tract protection may be important with regard to the control of acquired immune deficiency syndrome and other venereal diseases. It now appears possible that gastrointestinal, intraperitoneal and/or direct instillation of antigens into particular regions of the genital tract will elicit specific antibody responses against bacterial and viral antigens. Further studies are needed, however, to examine the role of oestradiol and progesterone during antigenic exposure, as well as at the time of antibody production, so that immune responsiveness may be optimized.

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