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IMMUNE REGULATION OF THE FEMALE REPRODUCTIVE TRACT

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INTRODUCTION

Historically the reproductive tract has been considered a part of the mucosal immune system. The main common features are the secretion of IgA by the polymeric Immunoglobulin Receptor (pIgR), the presence of Intra Epithelial Lymphocytes (IEL's) and the fine discrimination between tolerance and response. However, the reproductive tract has equally common features with the central compartment of the immune system; e.g. the preponderance of IgG as the effector isotype in the vagina and the absence of an organised reproductive mucosa associated lymphoid tissue that samples and processes the antigenic contents of the lumen of the reproductive tract. Recently several excellent reviews have been written on the immune defence in general of the female genital tract (*Parr* and *Parr*, 1999), on the hormonal influence of the immune response (*Wira* et al., 1999 and on animal models to study immunoprophylaxis (*Hook* et al., 1999).

This short review should be considered as complementary to these reviews and aims to emphasise some unique features such as the maintenance of a homeostatic vaginal flora by immunoregulation, the cyclic impermeability of the vaginal epithelia for sperm- and other antigens and a change in local lymphocyte mediated cytotoxicity during gestation.

It is not possible to discuss the immune defence of the vagina independently of the rest of the reproductive tract. The products of the ovaries, oviduct, uterus and cervix contribute substantially do the immune status of the vagina. Unique to the immunobiology of the reproductive tract are its multiple and apparent contradictory functions, for example, the defence against potential pathogens while maintaining the beneficial (homeostatic) flora of the vagina. Following each mating/insemination the local vaginal-cervical and/or uterine environment is compromised. The disturbances in the homeostatic flora should be restored quickly to prepare for potential implantation of the embryo(s). Implantation and gestation require a very unique contribution of the immune system which results in allogeneic tolerance while maintaining an acceptable level of xenogeneic immunity. While the special immunology of gestation will not be discussed here, allogeneic tolerance mechanism have to be maintained against sperm components. This particular tolerance seems to result mainly from the temporary impermeability of the vaginal/cervical epithelium and the immunosuppressive effect of sperm components (*Bronson* and *Fusi*, 1999; *Tristram* and *Ogra*, 1999)

Unique also to the immunology of the female reproductive tract is the cyclicity regulated by the secretion of oestrogens and progestagens, which change both morphology and functionality of the different regions of the tract.

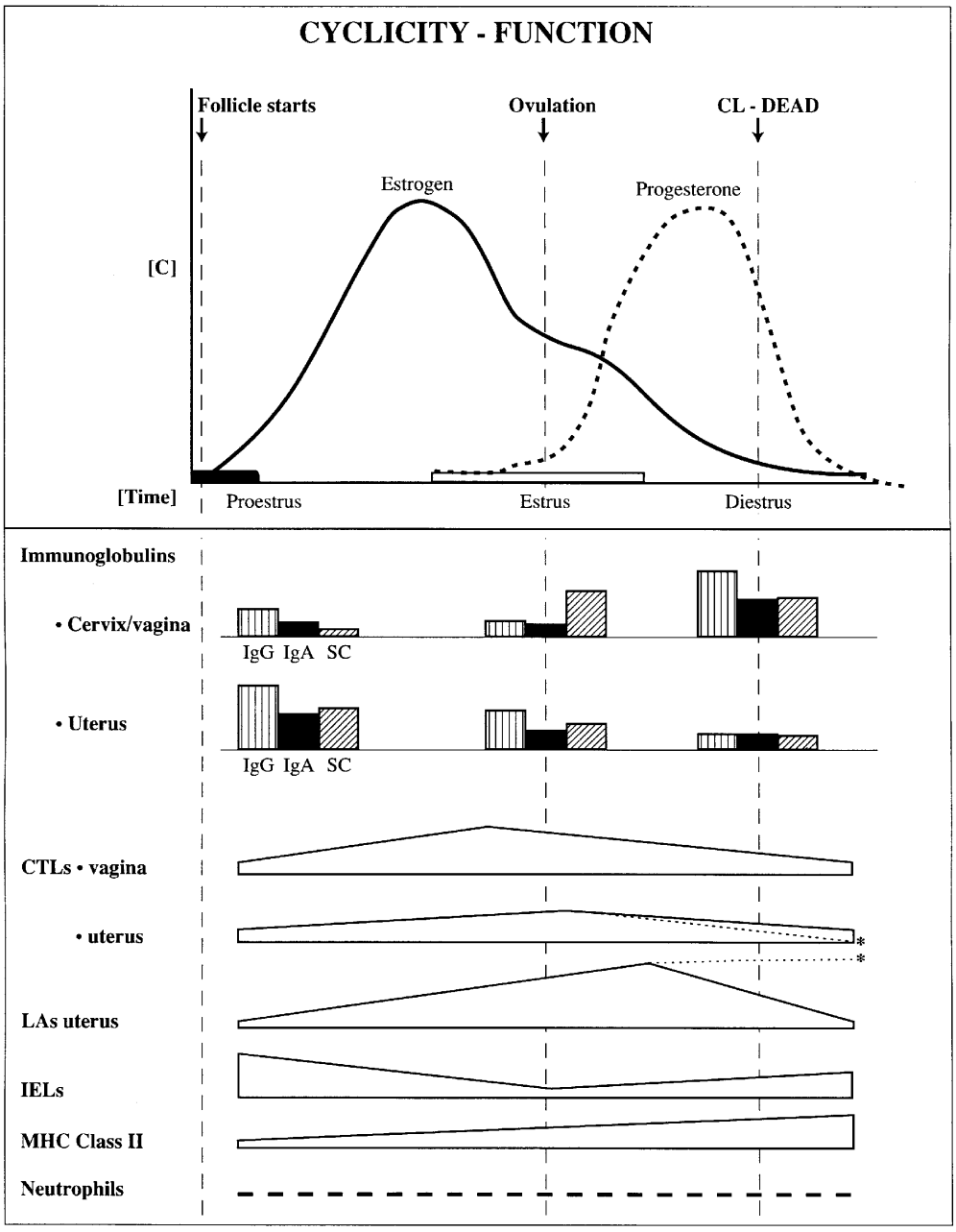

Figure 1: The relationship between the ovarian cycle and the relative abundance of immune components in the female reproductive tract.
Immunoglobulins: in general the secretions of the uterus are highest when they are lowest in the vagina.
SC (pIgR); secretory Component, this IgA transport molecule seems to be in excess in the non-inflamed reproductive tract.
CTL; Cytotoxic Lymphocyte. These cells peak around the time of ovulation. The CTLs disappear from the uterus when nidation and potential pregnancy is established (- - - - *).

Figure 1 depicts the ovarian hormonal cycle of a prototype mammal and the level of functionality, in the reproductive tract, of antibody isotypes and immune effector cells at different phases of the cycle. Very little data are available on the normal immune function of the vagina once gestation is established (*Tristram* and *Ogra*, 1999)

An additional source of complexity is the uniqueness of the reproductive strategy of the different mammalian groups, which is reflected in the different structures of the reproductive tract of the various species. The major experimental animals, the mouse and rat, have a somewhat similar copulation/insemination/fertilisation pattern. But even in this case it should be pointed out that the rat, unlike the mouse, has a very active entero-hepatic IgA recirculation and this might be the reason that hormone induced immunoglobulin secretion is preferentially studied in this species. Both genera utilise direct deposition of sperm to the cervix. In humans, by contrast, entero-hepatic IgA circulation is minimal, sperm is deposited outside the uterus and the cervix has a more active function in the uptake of the sperm cells. The structural result is that in the rat and mouse, vagina and cervix have a rather similar morphology and function, while in humans the cervix has become a centre of immune cells and antibody secretion (*Parr* and *Parr*, 1994). This is just one example of a different structure/function of the female reproductive tract among mammals. If one considers other groups such as swine, cows, horses and carnivores, it is not easy to reconstruct a common mammalian defence strategy of the reproductive tract. Figure 2 depicts the structure-function relationship of different parts of the reproductive tract of a prototype mammal.

Finally, we should mention the contribution of motility of the female reproductive tract to remove the ejaculate and hence the bulk of allogeneic and xenogeneic antigens. The importance of motility continues after fertilisation and little is known about its importance or connection with the immune system.

ORIGIN AND CONCENTRATION OF IMMUNOGLOBULINS IN VAGINAL FLUID

Ovary

The developing ovarian follicle produces a relatively large amount of follicular fluid. This fluid is very high in IgG (>10x serum concentration). The mechanism of this active transport is not very well studied, except that the IgG originates from the general circulation. Its function is also obscure and it is unknown if this fluid is largely taken up

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LA: Lymphoid aggregates; these tolerogenic structures are adjacent to developing endometrial glands. They increase towards the time of the arrival of the ovum (or zygote). When there is no pregnancy the LAs decrease towards the beginning of the next cycle. In case of pregnancy their number increases (- - - - *).

IEL; Intra Epithelial Lymphocyte.

MHC class II; as expressed on epithelial cells of vagina and cervix/uterus. An increase in class II expression is by some authors considered as an increase in antigen recognition and processing.. Peak class II expression occurs when the vaginal epithelium is thinnest and the access to the draining iliac lymph nodes is possible.

Neutrophils.; in the normal reproductive tract these cells are present in low numbers. However when luminal complement is activated. neutrophils can emigrate into the lumen in massive numbers. This luminal emigration can take place in a very short time period.

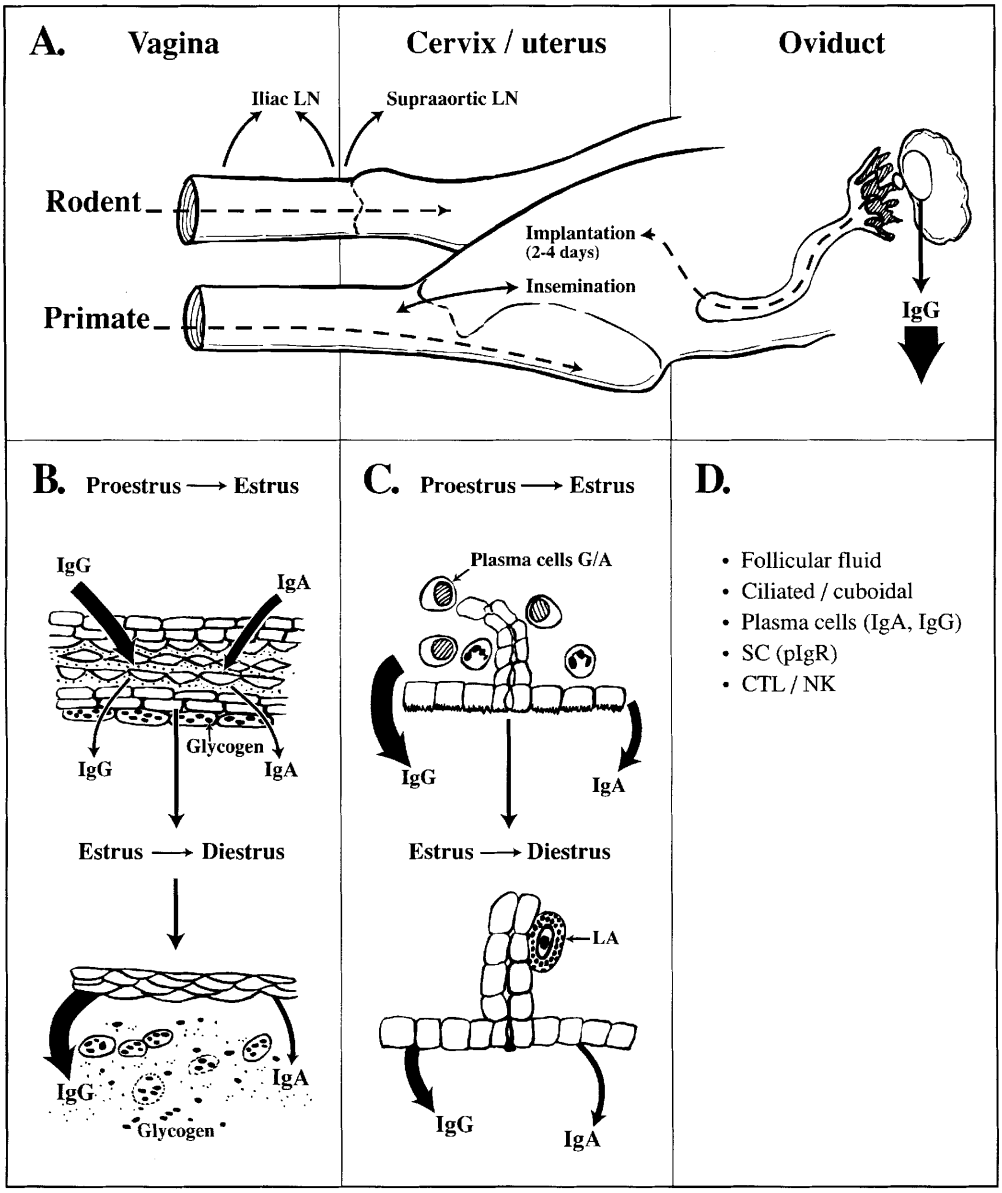

Figure 2: Structure-function relationship of the female reproductive tract.
A: Two different reproductive strategies are represented. Rodents with direct deposit of semen into the uterus and primates with deposition of sperm outside the uterus. As a result the immune components of the cervix are more pronounced in primates.
B: During the progression pro-oestrus oestrus, the squamous vaginal epithelium accumulates mainly IgG below the cells of the outer layers of the epithelium, these ageing cells also accumulate glycogen storage granules. During the progression from oestrus di-oestrus the outer layer of the epithelium is sloughed off, the cells disintegrate and allow the resident flora to expand utilising the released glycogen. During this expansion the local flora is selected for beneficial bacterial variants by the IgG that is released from the intercellular storage. At this time the vaginal epithelium is also very thin and now allows the draining of (bacterial) luminal antigens to the iliac lymph nodes.

by the infundibulum and serves as a defensive solution in which the ovum is transported to the uterus.

Oviduct

The data on the immune structures in the oviduct are few. It has been reported that the polymeric immunoglobulin receptor (pIgR) can be demonstrated on the apical sites of glandular and luminal epithelial cells. IgA plasma cells (although few) are present in the stroma and IgA and IgG can be demonstrated interstitially and in the apical regions of the epithelial cells. No data are available on the transport mechanism of IgG across the epithelium It is thought to be intracellular. Few data are available on the influence of the ovarian cycle on the immune parameters of the oviduct (*Wira* et al., 1999).

Uterus

During di-oestrus (high progesterone concentration) there is little contribution of immunoglobulins to the secretion from the uterus. When oestrogen increases during pro-oestrus there is a simultaneous increase in stromal IgG and IgA (*Richardson* and *Wira*, 1997). The increasing oestrogen concentrations enhance the transcription and translation of pIgR in glandular and luminal epithelial cells. Just before oestrus the secretion of sIgA and IgG is at its highest. At this time plasma cells (IgA) can be observed in the stroma, although not in the same amount as in the small intestine (*Wira* et al., 1999). At the time of oestrus this secretory phase has peaked, the expression of pIgR decreases with increased progesterone levels until the cycle is repeated after the disintegration of the corpus luteum. The main immunoglobulin in uterine secretions is IgG. The mechanism by which IgG reaches the apical site of epithelia is not well understood. Although IgA is generally lower than IgG, the uterus (and human cervix) are the main contributors of IgA in cervico-vaginal secretions.

Cervix

In rodents the epithelium of the ectocervix is similar in form and function to that of the vagina. In humans, however, both endo- and ecto-cervix act as major immune defence centres (*Parr* and *Parr*, 1999). The pIgR is under similar hormonal control as the in uterus. Some IgA and IgG plasma cells are observed in the cervix. While IgG is the predominant isotype in uterine secretions. The cervix and uterus contribute 90% of IgA that is found in cervico-vaginal secretions (*Kaushic* et al., 1997).

While some plasmacells are present in the normal, non inflamed tract, it is thought that the majority of secreted immunoglobulins is derived from the general circulation (*Hook* et al., 1999).

C: The epithelium of the cervix and uterus actively secretes IgG and IgA during the progression from di-oestrus pro-oestrus. These secretions reach the vaginal lumen at a time when immunoglobulin secretion by the vaginal epithelium is low.. During this time IgG and IgA plasma cells are most numerous as are granulocytes. During the progression oestrus di-oestrus immunoglobulin secretion is at its lowest in cervix and uterus, while it now reaches its peak in the vagina. At this time the Lymphoid Aggregates (LA) develop to prepare the uterus for its tolerogenic function in the event implantation takes place.
D: The ovary and oviduct also contribute to the secreted immunoglobulins, especially the follicular fluid).

The thickness of the arrows correlates with the relative abundance of the immunoglobulin isotypes.

Vagina

The timing of immunoglobulin secretion by the vaginal epithelium is basically complementary to the secretions by the uterus. When immunoglobulin secretion by the uterus is high during pro-oestrus (high oestrogen) the secretion is low in the vagina (*Kaushic* et al., 1994). The expression of pIgR follows the same pattern during pro-oestrus low in the vaginal epithelium and high in the uterine epithelium (*Wira* et al., 1999). When di-oestrus is reached (high progesterone) the sequence reverses and the concentration of IgG and IgA reaches a peak in the vaginal fluid while reaching its nadir in uterine secretions (*Parr* and *Parr*, 1994; *Parr* and *Parr*, 1999). This sequential timing of secretions gives the apparent picture that after mating the uterine environment is cleared of potential harmful antigens, first in the uterus followed in sequence by the immune clearance of the vagina. IgG is also the major isotype in vaginal secretions and the relative concentration of IgA is substantially less than in uterine secretions (*Parr* and *Parr*, 1999; *Rosenthal* and *Galichen*, 1997). Therefore the excess expression of pIgR might represent extra-transport capacity to be used during inflammation when blood supply and local plasma cell numbers increase, allowing for the rapid transport of the extra IgA The mechanism by which IgG reaches the vaginal lumen through the multilayer squamous epithelium is thought to occur in two phases (*Parr* and *Parr*, 1994). First IgG penetrates the basal layers via intercellular channels and accumulates below what is described as the granular layer. This granular layer consist of the top few layers of epithelial cells. These luminal cells contain large glycogen storage granules that are synthesised under increasing oestrogen concentrations. These cells are sloughed, release their glycogen that is converted to lactic acid by lactobacilli to create the optimum pH microconditions for the homeostatic vaginal flora. Simultaneously the IgG stored below the glycogen rich cells is now released and can act to neutralise pathogens but also contribute to the selection of the most beneficial of the vaginal flora in a manner described for the intestinal bacterial homeostasis by Rolf Freter many years ago (*Freter*, 1974). This time point coincides with the minimum thickness (layers) of the vaginal epithelium, the highest expression of Class II and permeability for non-self molecules (*Wira* and *Rossoll*, 1995) that can reach either actively or passively the draining iliac lymph node (*Prabhala* and *Wira*, 1995). This is the only time during the cycle that the vaginal epithelium is permeable for foreign antigens. It is also the time point when experimental infections with *Chlamydia* (*Kaushic* et al., 1998) and HSV-2 can be established successfully in rodents (*Parr* and *Parr*, 1998; *King* et al., 1998). It is apparent that any attempt to vaccinate via the intra vaginal route should take place during this phase of the cycle. Preliminary experiments indicate that during this phase foreign lymphocytes can penetrate the thin squamous epithelium and drain to the iliac node (*King*, 1998; *Rosenthal* and *Gallichan*, 1997). This observation lends credibility to the "Trojan Horse" hypothesis for heterosexual HIV transmission in the non-inflamed genital tract (*Ibata* et al., 1997; *Hook* et al., 1999; *Yeaman* et al., 1998).

DISTRIBUTION AND FUNCTION OF T-CELLS

T-cells are randomly distributed throughout the genital tract. They tend to be more numerous in the human cervix and rat vagina (*Givan* et al. 1997). A

linear correlation seems to exist between the number of T-cells and rising oestrogen levels (*White* et al., 1997a). However, this might merely reflect an increase in perfusion and total tissue mass of the tract. Of greater importance is their activity. When the total cytolytic activity of isolated T-cells is measured *in vitro* (CTL's), using an anti-CD3 monoclonal Ab, moderate cytolytic activity is found during the pre-secretion phase (high oestrogen-low progesterone) of the cycle (*White* et al., 1997b). It has been proposed by some investigators that these CTL's are important in inducing the secretory phase of the endometrium. However, once the secretory phase of the endometrium is reached (high progesterone-low oestrogen: oestrus => di-oestrus) the cytotoxic activity drops to non-detectable levels (*White* et al, 1997b). These observations also corroborate the hypothesis that a TH1 response capability is incompatible with implantation/nidation and that a TH2-response mode is required to accomplish this function successfully (*Yeaman* et al., 1998). Further evidence for this hypothesis is the observation that this CTL activity remains constant in post-menopausal women. However CTL activity remains constant in vagina and cervix (rat, mouse) during the entire ovarian cycle. The importance of CTL during infection is well proven by even a partial depletion *in vivo* of this T-cell population (*Parr* and *Parr*, 1998).

NATURAL KILLER CELLS (NK-CELLS)

NK cells are also randomly distributed throughout the tract in relatively large numbers. These cells are composed of three subgroups (mouse). The first group contains the characteristic LGL-1 membrane antigen and the absence of intracellular perforin. The second group is characterised by the presence of both the LGL-1 antigen and intracellular perforin. The third group lacks the membrane marker and contains large stores of perforin. The latter is the fully activated NK-cell phenotype. In the mouse the first type is present up to the 6th day of gestation and accumulates around the developing metrial glands. The second group becomes numerous by day six of gestation. Perforin producing cells become detectable by day 12 and the fully activated form by day 14 of gestation (*Parr* et al., 1991). Once established in the reproductive tract, these NK-cells seem to constitute a population that is separate from the circulating NK-cell pool. It has been suggested that NK-cells influence, like CTLs, the development of uterine gland and that the uterine stroma influences the maturation of the NKs (*Parr* et al., 1991). Thus it is plausible that NK-cells take over CTL-functions once the embryo is implanted.

ENDOMETRIAL GLAND LYMPHOID AGGREGATES

Small lymphoid aggregates are associated with the endometrial/cervical (human) glandular epithelium. The small aggregates consist of a core of B-cells surrounded by $CD8^+/CD4^-$ T-cells which in turn are surrounded by macrophage like cells. These aggregates develop during the high progesterone phase of the cycle and continue into pregnancy. Current opinion is that these aggregates have no "defence" function but act as tolerogenic centres to support the implantation of the allogeneic embryo. Unlike CTL activity these gland-

associated lymphoid aggregates are no longer present in the post-menopausal reproductive tract (*Yeaman* et al., 1997).

INTRA-EPITHELIAL LYMPHOCYTES (IELs)

IELs are very numerous in the epithelium of the reproductive tract. Similar to the intestine the larger majority have γT-Cell Receptor (TCR) (*Wira* et al., 1994). IEL numbers increase during di-oestrus (when the vaginal squamous epithelium is thinnest) followed by a decline during pro-oestrus and reaching the lowest numbers at the time of oestrus. Their exact function, like in the gastro-intestinal tract, is not well understood. The IELs are often associated with Langerhans cells in vagina and cervix and express CD4. In the cervix (human) and uterus the $CD8^+$ phenotype becomes most prevalent. It has been speculated that the IELs are influenced by interferon-γ (INF-γ) produced under high progesterone conditions by glandular and stromal epithelial cells. This is also the time of peak production of IL-6. The connection between INF-γ, IL-6 and IELs is not very well understood (*Wira* et al., 1999). Their increase during the latter part of the cycle might indicate that IELs either take over some CTL-function or have a tolerogenic function.

POLYMORPHONUCLEAR LEUKOCYTES (PMNs)

PMNs are well distributed throughout the healthy reproductive tract. However, during any form of infection they increase spectacularly and usually migrate in large quantities into the lumen of the tract. PMNs constitute a very effective and fast defence and “clean up” mechanism. Interestingly PMNs produce substantial amounts of INF-γ and seem to induce the epithelia to increase production of pIgR and the C3 component of complement by luminal epithelial cells, thus amplifying their own activity (*Givan* et al., 1997; *Yeaman* et al., 1998).

CONCLUSION

This short summary attempts to demonstrate how the individual immune components of the female genital tract interact and determine the microenvironment of the vagina. Because prophylaxis (immunisation) has a very high priority in both human and veterinary medicine, it is important to emphasise the following aspects:

1) The homeostasis of the vaginal flora seems to be maintained not by IgA, as in the intestine, but by IgG. The working hypothesis is that oestrogen induces glycogen production in the luminal layers of the vaginal squamous epithelium. When these cells slough and disintegrate the existing flora expands and at this time antigens of new strains/types can penetrate to iliac and supra-aortic lymph nodes where a preferentially IgG response is induced. This IgG accumulates during pre-oestrus under the granular layer (glycogen cells) and is released during the next cycle eliminating new or too dominating strains/types. Some authors claim that the mucosae-associated lymph-nodes such as the iliac and mesenteric nodes produce intrinsically

more IL-4 and hence tilt the outcome of the response somewhat toward an IgA response. However there is little firm evidence for this assumption and the general finding after experimental infections is that IgG constitutes indeed the major isotype of the response.

2) The reproductive tract is not the ideal place to induce a strong IgA response since no real Mucosa Associated Lymphoid Tissue (GALT) is connected with the genital tract. The epithelium of vagina, cervix and uterus seem to contain an extra capacity for the transport of IgA induced at a remote mucosal site of the upper respiratory or digestive tract , e.g. intra-nasal or rectal, (*Crowley-Norwick* et al., 1997) produces possibly sufficient IgA in the circulation to protect the surface of the reproductive tract. This has been demonstrated, e.g. by the intranasal administration of an adenovirus vector containing a glycoprotein (gB) of HSV-2 (*Galichen* and *Rosenthal*, 1996).

Consideration should also be given to the concept that a disturbance of the beneficial vaginal flora is likely to originate from pathogens residing in the digestive tract. The probability that these pathogens have previously been processed by the GALT is high ; resulting in a substantial IgA response/memory and the concomitant suppression of the IgG response. Thus in the case of invasion by a pathogen of intestinal origin there should exist a population of IgA memory cells , a circulating pool of specific IgA and likely a population of cells that suppress an IgG isotype response. The inflammation will induce mucosae specific addressins which in turn results in the homing of IgA committed cells to the reproductive mucosa (*Szabo* et al., 1997) and the large capacity of the pIgR will transport both locally produced IgA and circulating IgA into the lumen of the reproductive tract. This would explain the strong IgA response against the haemolysin of *Gardnerella vaginalis* when this pathogen infects the vagina (*Gauci*, 1999).

3) Contrary to paradigms it is not a requirement for protection of the genital tract that an IgA response is induced given the fact that IgG is the dominant isotype and antibody in vaginal secretions. The timing of antigen application, however, seems to be crucial because access to the iliac lymph nodes seems only possible towards the end of the ovarian cycle. The feasibility of this route of immunisation when the timing in the ovarian cycle is observed is proven by experiments using attenuated HSV-2 in progesterone treated rats (*Galichen* and *Rosenthal*, 1996).

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