



Review

The potential use of sperm antigens as targets for immunocontraception; past, present and future

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Abstract

Immunocontraception, and in particular the targeting of antibodies to gamete-specific antigens implicated in sperm–egg binding and fertilisation, offers an attractive approach to the growing global problem of overpopulation. Such an idea is not new; indeed several immunocontraception trials, using animal model systems, have been reported in recent years and a number are reviewed here. However, the results of these studies have been largely disappointing. We believe that two fundamental flaws attribute to the poor success of most of these preliminary immunocontraceptive trials. Firstly, loss of fertility has invariably been used as the assay. This presupposes that immuno-neutralisation of a single, gamete-specific antigen will be sufficient to cause a significant reduction in fertility; however, recent data suggests that such a premise may not be well-founded for a number of reasons. Secondly, and arguably the most important flaw, is the almost universal, but largely inappropriate, use of systemic immunisation as the sole route of antigen delivery. Whilst systemic immunisation regimes may lead to high serum IgG levels, these levels do not correlate with specific antibody levels in the reproductive tract or with contraceptive efficacy. Hence, an alternative antigen delivery approach is required which will induce an effective local immune response in the reproductive tract. Here we discuss the ways in which this might be achieved. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

At the present growth rate, the world population is estimated to reach a phenomenal 10 billion people by the year 2050, presenting a real risk of overpopulation with grave implications for the future. That new forms of contraception are required is indisputable, as many of those currently available carry the risk (albeit low) of unacceptable side effects, may be inappropriate or unavailable for a proportion of the population due to personal, cultural or financial reasons or may not provide adequate protection against pregnancy. A novel contraceptive strategy that is receiving considerable attention is that of immunocontraception; the use of antifertility vaccines to prevent fertilisation.

As with conventional vaccines, contraceptive vaccines utilise the body's hormonal defence system, but rather than preventing pathogenic infection, such vaccines are designed to block an indispensable step in the reproductive process. A successful contraceptive vaccine must meet a number of criteria; it must be safe, reliable, affordable, easy to administer, capable of evoking an homologous response in all or most individuals, confer a high level of contraceptive efficacy and be acceptable to a wide range of the population. Ideally, such a vaccine should provide a contraceptive effect that is both long lasting and reversible, with the predicted immune response easily determined by a simple diagnostic test. Finally, the application of contraceptive vaccines are by no means restricted to the control of human fertility; similar approaches are being developed for the control of pest species such as the European rabbit and red fox in Australia.

An obvious immunocontraceptive strategy is to select target antigens with established roles in gamete interactions. One such antigen that has received considerable attention is ZP3, a major glycoprotein of the zona pellucida with sperm receptor function. Immunisation of female subjects with native ZP3 interferes with fertility in a number of mammalian species (Gupta et al., 1997), but is frequently associated with alterations in hormonal profiles and follicular development. Similarly, immunisation with synthetic peptides of ZP3 can result in induction of autoimmune oophoritis and ovarian dysfunction (Rhim et al., 1992; Skinner et al., 1984), although appropriate modification of the immunogen can avoid pathogenic T-cell responses whilst maintaining specific antibody production (Lou et al., 1995). In the male, immunisation of guinea-pigs with the sperm surface protein PH-20 leads to the induction of experimental autoimmune orchitis (Tung et al., 1997). Both of these studies clearly emphasise the problems associated with induction of autoimmune responses. In view of the above, an arguably more attractive approach is to immu-

nise female subjects with sperm-specific antigens thereby avoiding such potentially pathogenic autoimmune responses.

The rationale and feasibility of using sperm-specific proteins as immunocontraceptive targets derives from the well documented antifertility effect of naturally occurring anti-sperm antibodies in infertile couples (Bronson et al., 1984; Shulman, 1986). Most individuals do not develop an immune response to sperm antigens, even though repeatedly exposed. However, the immunogenicity of sperm is clearly demonstrated by the presence of anti-sperm antibodies in up to 70% of vasectomised men, probably as a result of leakage of sperm into the abdominal cavity following vasectomy and/or the epididymal resorption of millions of trapped spermatozoa (Alexander, 1972). Examination of the epididymal ducts of such men indicates that many phagocytic cells infiltrate to engulf and degrade spermatozoa (Marsh and Alexander, 1982). Similarly, the presence of anti-sperm antibodies in females, although infrequent, is often associated with the concomitant presence of a sexually transmitted disease (Cunningham et al., 1991), acting as an adjuvant and evoking an inflammatory response in the reproductive tract. The antigenic determinants on sperm against which such antibodies are generated have not been extensively characterised and documented, although as discussed below screening with such antibodies has been used in an attempt to identify potential targets for immunocontraceptive evaluation. However, the detrimental effect of such antibodies on fertility is primarily caused by their ability to trap, or agglutinate, sperm in semen and cervical mucus rather than by specifically inhibiting gamete interactions.

Whilst the generation of naturally-occurring anti-sperm antibodies demonstrates the viability of immunocontraception as an effective strategy, the use of poorly-characterised, crude sperm extracts as immunogens is not a particularly practical or safe basis for vaccine development. A much better approach would be one which exploits well characterised sperm antigens, exclusively expressed by spermatogenic cells.

For a sperm protein to be considered as a potential immunocontraceptive target antigen, it needs to fulfil a number of important criteria: (1) ideally, the antigen must be exclusively expressed by spermatogenic cells (with no cross-reacting immunogenic epitopes on somatic cell components); (2) the antigen needs to be exposed on the surface of sperm to enable antibody recognition; and (3) it is assumed, at least at present, that such an antigen should have an essential role in sperm function, although other abundant proteins exclusively expressed on the surface of sperm may also prove to be appropriate targets (e.g. by invoking sperm agglutination).

2. Sperm proteins with potential as immunocontraceptive target antigens

To date, a number of sperm-specific proteins with immunocontraceptive potential have been identified by screening panels of antibodies generated by the immunisation of rodents with whole sperm extracts. Of these, only a small number have been well documented (Table 1) and shown to be involved in sperm-zona recognition (SP10, SP17 [or RSA 3], FA-1 and PH-20 [or 2B1]) or sperm–oolemma membrane interactions (fertilin α and β and cyritestin [or tMDC I]). Other reported sperm antigens which may warrant further investigation include a 26kDa hamster sperm glycoprotein (Berube and Sullivan, 1994), mouse NZ-1 (Naz and Zhu, 1997), RSA 1 (O’Rand and Porter, 1982; O’Rand et al., 1993), mouse and porcine zonadhesin (Hardy and Garbers, 1994; Gao and Garbers, 1998), equatorin (Toshimori et al., 1998) and SOB2 (Lefevre et al., 1997), all of which are exclusively expressed by spermatogenic cells and have been shown to be involved in zona or oolemma membrane interactions (see Table 1). A further sperm-specific antigen (human SIAA) recognised by monoclonal antibody I9G9, which inhibits sperm penetration of zona-free hamster eggs (Jimenez et al., 1994), may also be worthy of consideration.

SP10 is a sperm-specific acrosomal protein that was first identified in the human using a monoclonal antibody (Herr et al., 1990a) and subsequently cloned and sequenced in human (Wright et al., 1990, 1993), mouse (Reddi et al., 1995), fox (Beaton et al., 1995), baboon and macaque (Freemerman et al., 1993). Immunological studies have also identified SP10 on bovine and porcine sperm (Herr et al., 1990b; Coonrod et al., 1996). Antibodies to SP10 inhibit bovine in vitro fertilisation by reducing sperm-zona secondary binding (Coonrod et al., 1996) and a monoclonal antibody HS-63, which was subsequently found to also recognise the mouse orthologue of SP10, inhibits mouse in vitro fertilisation (Liu et al., 1989). Both of these antibodies also inhibit human sperm penetration of zona-free hamster eggs (Anderson et al., 1987; Liu et al., 1989). Although the contraceptive efficacy following immunisation with SP10 has not yet been fully evaluated in any species, specific anti-SP10 antibodies have been measured in vaginal secretions of mice following oral immunisation with attenuated *Salmonella* sp. expressing human SP10 (Srinivasan et al., 1995) and in oviductal fluid of macaques following intramuscular immunisation (Kurth et al., 1997). Antibody levels in the oviduct are of particular relevance since SP10 is localised within the acrosomal compartment (Foster et al., 1994) and the outer acrosomal membrane complex (Olson et al., 1997) and is therefore only accessible to antibody after the acrosome reaction has been initiated.

SP17 is a sperm-specific protein that was originally isolated as a member of the rabbit sperm autoantigen (RSA) family (O’Rand and Porter, 1982),

Table 1
Properties of sperm surface antigens implicated in sperm–egg interactions

Protein	Species	Seq ^a	Final location of mature protein	Binds to	inhibition of binding ^b	
					In vitro	In vivo
Fertilin β	Guinea-pig	Yes	Posterior head	Oolemma plasma membrane	Yes	Poor
	Rat	Yes	Acrosomal membrane			
	Mouse	Yes	Equatorial region & inner acrosomal membrane			
	Rabbit	Yes	Head			
	Bovine	Yes	Posterior head			
	Macaque	Yes	n.d.			
Fertilin α	Human	Yes	n.d.	Oolemma plasma membrane	Yes	n.d.
	Guinea-pig	Yes	n.d.			
	Rat	Yes	n.d.			
	Mouse	Yes	n.d.			
	Rabbit	Yes	Whole head			
	Macaque	Yes	n.d.			
tMDC I/ cyritestin	Human	Yes	Non-functional	Oolemma plasma membrane/equatorial segment ^c	Yes	n.d.
	Mouse	Yes	Inner acrosomal membrane			
	Rat	Yes	n.d.			
	Macaque	Yes	n.d.			
PH-20/2B1	Human	Yes	Non-functional gene	Penetration of cumulus via hyaluronidase activity; 2° zona binding	Yes	Yes
	Guinea-pig	Yes	Plasma & inner acrosomal membrane of head			
	Rat	Yes	Acrosomal			
	Mouse	Yes				
	Rabbit	Yes		Head, plasma- & inner acrosomal membrane		
	Macaque	Yes				

Table 1 (Continued)

Protein	Species	Seq ^a	Final location of mature protein	Binds to	inhibition of binding ^b	
					In vitro	In vivo
SP10	Human	Yes	Head, plasma- & inner acrosomal membrane			
	Human	Yes	Equatorial & acrosomal membrane	2° zona binding	Yes	n.d
	Mouse	Yes	Intra-acrosomal membrane			
	Baboon	Yes	Intra-acrosomal membrane			
	Macaque	Yes	n.d.			
	Fox	Yes	n.d.			
RSA family ^d	Bovine	No	OMC & outer acrosomal membrane			
	Porcine	No	n.d.			
	Rabbit	No	n.d.		Yes	Yes
SP17/RSA ₃ ^c	Mouse	Yes	Equatorial segment, principal & mid-piece	ZP3 carbohydrate moieties		Yes ^f
	Macaque	Yes	Plasma membrane principal piece			
	Human	Yes				
	Baboon	Yes				
	Rabbit	Yes		(R45 & R55 in rabbit)		
FA-1	Human	Part	Post acrosomal plasma membrane	ZP3	Yes	Yes
	Mouse	Yes	n.d			
	Rabbit	No	n.d			
	Bovine	No	Post acrosomal			
	Macaque	No	n.d			
P26H	Hamster	No	Plasma membrane & acrosome	Zona	Yes	Yes
NZ-1	Mouse	Yes	n.d.	Zona		
SIAA	Human	No	Intra-acrosomal membrane	n.d.	Yes	n.d

Table 1 (Continued)

Protein	Species	Seq ^a	Final location of mature protein	Binds to	inhibition of binding ^b	
					In vitro	In vivo
Zonadhesin	Mouse	Yes	n.d.	Zona	n.d.	n.d.
	Porcine	Yes	n.d.			
Equatorin	Mouse	No	Equatorial	Oolemma plasma membrane	Yes	n.d.
	Rat	No	Equatorial			
	Human	No	Equatorial			
SOB2	Human	No	Post-acrosomal & neck	Oolemma plasma membrane	Yes	n.d.
Prot. DE/ AEG ^e	Rat	Yes	Dorsal head & equatorial segment	Oolemma plasma membrane	Yes	Yes
gp20 ^e	Human	No	Equatorial	Oolemma plasma membrane	Yes	n.d.
EP140 ^e	Rabbit	Part	Acrosome & midpiece plasma membrane	n.d.	n.d.	n.d.
LDH-C4	Baboon	Yes	Sperm tail	Causes sperm agglutination	n.d.	Yes
	Fox	Yes				
	Mouse	Yes				

^a 'Seq' indicates whether the complete amino acid sequence of the sperm protein has been determined (normally deduced from the cloned cDNA sequence).

^b 'Inhibition of binding in vitro' refers to the use of specific antisera, peptide mimics or recombinant proteins in sperm-egg binding studies. 'Inhibition of binding in vivo' refers to immunisation trials in whole animals.

^c Cyritestin has been alternatively localised to the inner acrosomal membrane (Linder et al., 1995) or the equatorial segment (Yuan et al., 1997) in two independent studies.

^d The RSA (rabbit sperm autoantigen) family consists of three antigens; RSA 1, RSA 2 and RSA 3. Inhibition of binding studies, both in vitro and in vivo, have used a mixture of all three antigens. Additional data is available on RSA 3 alone (also known as SP17).

^e Protein DE (also known as AEG), gp20 and EP140 are all epididymal secretory proteins which associate with the sperm surface during epididymal transit.

^f The observed reduction in fertility in vivo was dependent on the strain of mouse.

antibodies to which have been shown to impede fertilisation both in vitro and in vivo (O'Rand 1981; O'Rand et al., 1984). SP17 was subsequently cloned and sequenced in the human (Lea et al., 1996), baboon (Adoyo et al., 1997), macaque (Lea et al., 1998a,b), rabbit (Richardson et al., 1994) and mouse (Kong et al., 1995). In the mouse, SP17 is only exposed on the surface of sperm following the acrosome reaction (Kong et al., 1995), after which it is found throughout the equatorial segment where it has been shown to bind the carbohydrate component of the zona pellucida (O'Rand et al., 1988); in the rabbit, it specifically binds to ZP3 (proteins R45 and R55; Yamasaki et al., 1995). Immunisation of female mice with a chimeric peptide composed of SP17 and a promiscuous T-cell epitope, led to a reduction in fertility. However, this reduction did not correlate with either serum or vaginal antibody levels and, somewhat surprisingly, the response was dependent on the strain of mouse (Lea et al., 1998a,b).

FA-1 is a sperm-specific glycoprotein originally isolated from human and murine sperm and subsequently also found to be expressed on sperm from rabbit, bull and macaque. The cDNA encoding murine FA-1 has been cloned and sequenced (reviewed in Naz, 1996). Antibodies to FA-1 inhibit in vitro fertilisation in all of the above species, by interfering with sperm–zona interactions (Naz et al., 1992) and immunisation of female rabbits and mice with FA-1 does appear to reduce fertility in vivo (Naz, 1987; Naz and Zhu, 1998).

The sperm surface PH-20 antigen has been cloned and studied in a number of species including the guinea-pig (Lathrop et al., 1990a), rat (where it is known as 2B1; Hou et al., 1996), mouse (Lathrop et al., 1990b), macaque and human (Lin et al., 1993). PH-20/2B1 antigen appears to be a bi-functional sperm plasma membrane protein. Firstly, its hyaluronidase activity allows acrosome-intact sperm to penetrate the cumulus cell layer surrounding the oocyte (Lin et al., 1994) and secondly, it appears to be required for acrosome-reacted sperm to bind to the zona pellucida (Primakoff et al., 1988a; Shalgi et al., 1990; Hunnicutt et al., 1996). Antibodies raised against PH-20 and 2B1 significantly reduce sperm–zona binding in vitro (Primakoff et al., 1985; Shalgi et al., 1990) and in vivo immunisation trials in both male and female guinea-pigs were reported to lead to infertility in all immunised animals (Primakoff et al., 1988b). However, it has subsequently been shown that autoimmune orchitis was induced in the male guinea-pigs with the resultant infertility attributable to the absence of sperm in the epididymis (Tung et al., 1997). Somewhat surprisingly, the promising preliminary results obtained with female guinea-pigs does not appear to have been further followed up.

Two further sperm surface proteins, a testis-specific β 1-4 galactosyl transferase (Lu and Shur, 1997) and sp56 (Cheng et al., 1994), both of

which are reported to bind to carbohydrate moieties on ZP3, have been proposed to play a role in sperm–zona binding (for discussion, see Johnston et al., 1998). Whilst these proteins are also possible contraceptive targets, the finding that the testis-specific galactosyl transferase isoenzyme and its somatic cell counterpart are identical, except for a C-terminal cytoplasmic extension on the former, makes this an unlikely immunocontraceptive target.

Finally, although not directly involved in gamete interactions, the testis-specific isoform of lactate dehydrogenase (LDH-C4), or synthetic peptides derived from it, have been the subject of a number of immunocontraceptive trials over the past decade. Some of these trials have produced encouraging results (O'Hern et al., 1995, 1997), although the immunocontraceptive effect achieved has generally been attributable to sperm agglutination rather than interfering with gamete interactions.

3. The MDC protein family

The MDC proteins are a rapidly growing family of integral membrane proteins, all of which contain a number of distinct conserved features including a Metalloproteinase-like domain, a Disintegrin-like domain, a Cysteine-rich domain, a pro-domain and a transmembrane domain (also known as the ADAM family; proteins with A Disintegrin And Metalloproteinase domain); although recently a secreted MDC protein lacking a transmembrane domain, ADAMTS-1, has been reported (Kuno et al., 1997). A number of MDC proteins are found to be expressed within the mammalian testis (Wolfsberg et al., 1995; Howard et al., 1996; Krätzschar et al., 1996; Weskamp et al., 1996; Black et al., 1997; Frayne et al., 1997, 1998) with an increasing number detected on spermatogenic cells (Wolfsberg et al., 1995; Linder et al., 1995; Frayne et al., 1997, 1998), suggesting a role in reproductive function. Indeed, the significant sequence similarity of these proteins to a number of snake venom haemorrhagic proteins involved in integrin binding, led to the original postulation that the MDC proteins may also be involved in integrin binding via an XCD tripeptide within their disintegrin-like domain. Such an hypothesis is supported by the expression of a number of integrins on the oolemma plasma membrane (Fusi et al., 1993; Tarone et al., 1993; Almeida et al., 1995; Evans et al., 1995).

Fertilin α and β (collectively known as fertilin) and cyritestin (also known as tMDC I) are all members of the MDC protein family which have been well characterised and for which specific roles in sperm–egg interactions have been proposed.

Fertilin α and β were first described in the guinea-pig where they were reported to exist as a sperm membrane heterodimeric complex (Blobel et al., 1990), with the β subunit implicated in sperm–egg binding and the α subunit in sperm–egg membrane fusion mediated via a putative fusogenic peptide within the cysteine-rich domain. Fertilin α and β have now been cloned and sequenced from a number of species including guinea-pig (Blobel et al., 1992), mouse (Wolfsberg et al., 1995), rat (Frayne et al., 1997), bovine (Waters and White 1997), rabbit (Hardy and Holland, 1996), macaque (Perry et al., 1995) and human (Gupta et al., 1996; Vidaeus et al., 1997); cyritestin/tMDC I has been cloned in the mouse (Wolfsberg et al., 1995), rat (Frayne et al., 1997), macaque (Barker et al., 1994) and human (Frayne and Hall, 1998).

In rodents, both fertilin β and cyritestin are exclusively expressed by spermatogenic cells (Wolfsberg et al., 1995; Frayne et al., 1997; Lemaire et al., 1994), whereas in the macaque, fertilin β is sperm-specific but cyritestin is also expressed in a range of other tissues (Frayne et al., 1998). Fertilin α , is also now known to be expressed in a range of tissues in both rodents (Wolfsberg et al., 1995; Frayne et al., 1997; McLaughlin et al., 1997) and macaques (Frayne et al., 1997). Furthermore, both the human fertilin α gene and the human cyritestin gene are non-functional (Jury et al., 1997, 1998; Frayne and Hall, 1998), illustrating the danger of using rodents as models of human/primate fertility. Whilst such a result might negate the use of cyritestin as an immunocontraceptive target in primates it may still be an attractive candidate in non-primate species.

Cyritestin has been reported by Linder and co-workers (1995) to be located exclusively on the inner acrosomal membrane of murine spermatozoa by immunocytochemistry and electron microscopy (Forsbach and Heinlein, 1998). However, Yuan et al. (1997) dispute this localisation, instead showing cyritestin to be restricted to the equatorial segment of murine sperm, a distribution pattern that was unchanged following the acrosome reaction. The reason for this discrepancy is not clear. The localisation of fertilin β also appears to vary, particularly between different species; in the rat fertilin β has been localised to the inner acrosomal membrane (McLaughlin et al., 1997), in the mouse to the equatorial region (Yuan et al., 1997), in the rabbit to the entire head region (Hardy et al., 1997) and in the guinea-pig to the posterior head region of mature spermatozoa (Primakoff et al., 1987). Few studies have specifically localised fertilin α , but in the rabbit and guinea-pig the localisation would appear to be similar to that of fertilin β (Primakoff et al., 1987; Hardy et al., 1997). Cyritestin, fertilin α and fertilin β are all post-translationally processed by endoproteolytic removal of their N-terminal portions leading to membrane-bound proteins retaining the disintegrin-like, cysteine-rich and extracellular domains on

mature fertilisation-competent sperm in the cauda epididymidis (Linder et al., 1995; Yuan et al., 1997; McLaughlin et al., 1997; Forsbach and Heinlein, 1998; Frayne et al., 1998). Indeed, many sperm surface proteins that undergo post-testicular processing are proposed to be important for gamete interactions during fertilisation; in some cases processing correlates with the acquisition of fertilising ability (Jones et al., 1996), possibly suggesting a role for such processing in unmasking binding domains.

Evidence for a role for fertilin α in sperm–egg interactions and/or membrane fusion is far from conclusive. In one study, peptide mimics of the disintegrin-like domain of fertilin α appeared to exert limited inhibition of sperm–egg membrane binding (Yuan et al., 1997), although the significance was difficult to establish. However, in a subsequent study, the extracellular domain of recombinant fertilin α , with or without the disintegrin domain, specifically inhibited sperm–egg binding but had no effect on membrane fusion (Evans et al., 1998).

Evidence for a functional role for fertilin β and cyritestin in sperm–egg binding stems from studies using peptide mimics of the disintegrin-like loop which were shown to significantly inhibit sperm–egg interactions in vitro (Myles et al., 1994; Almeida et al., 1995; Evans et al., 1995; Yuan et al., 1997; Linder and Heinlein, 1997). Further studies using a recombinant fertilin β protein (representing the extracellular domain) have implicated fertilin β in interacting with a $\beta 1$ integrin on the oolemma plasma membrane (Evans et al., 1997). Whilst sperm from a fertilin β knockout mouse (Cho et al., 1998) showed reduced oolemma binding in vitro as anticipated, the observed reduction in fertility in vivo was largely attributed to a reduction in the number of sperm reaching the oviduct. A further anomaly was the inability of the fertilin β knockout sperm to bind to the zona in vitro. However, in vivo immunisation trials with fertilin β have as yet proved disappointing (Ramarao et al., 1996).

4. Epididymal secretory proteins

A number of glycoproteins on the surface of spermatozoa are acquired from epididymal secretions during transit through the epididymis. Although many such proteins may have either a protective role (e.g. glutathione peroxidase type 5 and extracellular superoxide dismutase; Williams et al., 1998a,b) or a modulatory role in sperm maturation (Orgebin-Crist and Fournier-Delpech, 1982; Cuasnicu et al., 1984a,b; Gonzalez-Echeverria et al., 1984; Moore et al., 1987), some have been specifically implicated in sperm–egg interactions at fertilisation, e.g. human gp20 (Focarelli et al., 1998) and rat protein DE (Cuasnicu et al., 1990). Of these the latter is the most well characterised.

Protein DE (also known as acidic epididymal glycoprotein, AEG) is a 37kDa glycoprotein that associates with the dorsal region of the sperm head during epididymal maturation (Kohane et al., 1980a,b) with binding characteristic of a receptor-type mechanism (Hall et al., 1996; Hall and Tubbs, 1997). The protein then migrates to the equatorial segment during both in vitro and in vivo acrosome reaction (Rochwerger and Cuasnicu, 1992). Antibodies to protein DE/AEG significantly inhibit penetration of zona-free eggs in vitro (Cuasnicu et al., 1990), and in vivo fertilisation rates are significantly reduced following artificial insemination with sperm previously exposed to antibody (Hall and Tubbs, 1997) or in male rats immunised with purified protein DE/AEG (Ellerman et al., 1998). Similarly, sperm–egg fusion is significantly reduced following exposure of zona-free eggs to purified protein DE (Rochwerger et al., 1992; Cohen et al., 1996).

5. Possible problems in eliciting an effective immunocontraceptive response

It is evident from the above and similar studies that although antibodies or peptide mimics of sperm-specific antigens have proved to be extremely effective at reducing sperm–egg interactions in vitro, immunisation trials have been largely unsuccessful in terms of contraceptive efficacy. The poor success of such trials are most likely attributable to both the target antigens used and the route of immunisation.

Firstly, all of these studies used loss of fertility as their assay, with the inherent assumption that immuno-neutralisation of a single sperm antigen may be sufficient to cause a significant reduction in fertility. However, as is evident from the literature, there are a number of determinants expressed by spermatozoa that have been implicated, at least in vitro, in sperm–egg interactions. Whether such proteins participate at fertilisation in a sequential manner, a co-operative manner, or whether some of these proteins are redundant, has yet to be firmly established. However, for most sperm surface proteins implicated in sperm–egg interactions, specific antisera, peptide mimics and recombinant proteins may significantly reduce, but do not completely block these interactions in vitro, suggesting that they are unlikely to play an essential role. Instead we propose that many of these sperm proteins act co-operatively, implying that the concerted effects of several proteins will lead to ‘maximum fertilising ability’, but that individual proteins are functionally redundant and are therefore not absolutely essential. Nevertheless, the co-operative nature of these interactions would mean that loss of individual proteins might lead to a concomitant reduction in ‘fertilising ability’, a situation which is arguably supported by our recent finding of non-functional fertilin α (Jury et al., 1997, 1998) and tMDC I/cyritestin (Frayne and Hall, 1998) genes in man.

Consequently, a successful immunisation regime might need to target not one, but a combination of sperm antigens. A possible regime for initially determining the *in vivo* contraceptive efficacy of selected sperm antigens may be to mix the appropriate antibodies found to be effective *in vitro* with a defined volume of semen, followed by insemination of females. Such an approach was found to be effective for testing the contraceptive effect of several rabbit sperm-agglutinating monoclonal antibodies (Castle et al., 1997).

Secondly, it is arguable that the major problem with such studies is the almost universal, but largely inappropriate, use of systemic immunisation as the sole route of antigen delivery. Whilst such immunisation regimes have been shown to generate high levels of circulating IgG antibodies, these do not correlate with contraceptive efficacy or specific antibody levels in the reproductive tract. Hence, an alternative antigen delivery approach is required which will induce an effective local immune response in the reproductive tract before the contraceptive efficacy of sperm antigens can be reliably evaluated.

6. The local secretory immune system of the female reproductive tract

The female reproductive tract is part of the secretory immune system which utilises secretory IgA as the predominant immunoglobulin in external secretions. Hence it is endowed with a significant number of plasma cells, mainly producing the IgA isotype, although IgG- and IgM-producing cells are also present (Kutteh et al., 1988, 1990; Parr and Parr, 1994). The majority of IgA produced by these plasma cells also contains a J chain; clearly suggesting that most of the IgA produced in these regions is largely polymeric (pIgA). In addition, the epithelial cells lining the fallopian tubes, the body and fundae of the uterus, and the cervix, produce secretory component; the polymeric Ig receptor which is required for transport of pIgA into secretions of the reproductive tract.

In humans, the majority of plasma cells are present within the endocervix, followed by the ectocervix and fallopian tubes, with relatively few cells present in the vagina (Kutteh et al., 1988, 1990; Parr & Parr, 1994). In contrast, several studies have reported only quite rare and scattered Ig-producing cells in the normal human endometrium (Tourville et al., 1970; Kelly and Fox, 1979; Kutteh et al., 1988). In view of the congruent distribution of secretory component and IgA throughout the endometrial epithelium, particularly in the glands (Brandtzaeg et al., 1993; Bjercke and Brandtzaeg, 1993), this would indicate that serum is an important source of pIgA in this region of the tract, derived via the rich vascular bed present in the

superficial layer of the endometrium (Brandtzaeg, 1997). Similarly, plasma cells are also rare in the endometrium of other species, including the rat (Mitchell, 1986; Parr and Parr, 1989), but paradoxically are abundant in this region of the murine uterus, as reviewed in Parr and Parr (1994).

The female reproductive tract therefore exhibits characteristics of a mucosal effector site with considerable pIgA production coupled with active transfer of both locally-produced and serum-derived pIgA. In addition, paracellular diffusion of locally-produced and serum-derived IgG through the epithelia appears to be an important part of the humoral immune system in this tissue (Brandtzaeg, 1997). Finally, the levels of immunoglobulins, secretory component and antibody secreting cells have all been clearly shown to be increased by oestradiol in the mouse and rat (Rachman et al., 1983; Wira et al., 1994). Thus, although systemic immunoglobulins may contribute to antibody levels in the female reproductive tract, effective immunocontraception directed against sperm-specific antigens will necessitate that regimes induce efficient local immune responses in the reproductive tract, rather than simply generating high levels of circulating antibodies. Indeed, a combination of both mucosal and systemic immune responses may be essential for generating an optimal response in the reproductive tract.

7. Immunisation routes

To date, studies on the local immune system of the female (and male) reproductive tract have been largely neglected by both immunologists and reproductive biologists. Nevertheless, a small number of published studies have reported antibody levels in vaginal fluids using a variety of non-gamete-derived antigens and a number of alternative immunisation routes. However, whilst in any single study antibody levels often varied, depending on the immunisation route used, comparisons between different studies, carried out by different groups, often yielded conflicting results. For example, immunisation via the vagina with the same antigen resulted in an antibody response in vaginal secretions in some studies (Johansson et al., 1998), whereas in other studies no response was detected (Haneberg et al., 1994).

The female reproductive tract is part of the common mucosal immune system (CMIS), along with the gastrointestinal (GI) and respiratory tracts. In the GI tract, primed immune cells from the regional lymph nodes preferentially home back to the GI tract, brought about by the expression of specific 'homing receptors' (Butcher and Picker, 1996). Furthermore, there is good evidence that the corresponding mucosal homing determinants

are shared among the Peyer's patches, the mesenteric lymph nodes and the intestinal lamina propria (Salami and Jalkanen, 1991), although a certain fraction of cells will end up in mucosal tissues and exocrine glands outside of the gut. Hence, by analogy with the GI tract, maximum immune responses in the reproductive tract should be achieved by strategies that induce activation, proliferation and differentiation of immune cells in the respective draining lymph nodes; the iliac lymph nodes in the case of the reproductive tract. In this context it is interesting to note that the distribution of IgA1: IgA2 cells found in the human reproductive tract is similar to that seen in the large intestine (McGhee et al., 1994) which utilises the same regional lymph nodes, supporting the concept that immune cells stimulated in these lymph nodes preferentially home to the reproductive sites. Indeed, immunisation of such nodes in the mouse has been shown to induce a significant immune response in reproductive tract secretions (Thaper et al., 1990) and studies targeting these lymph nodes in the cat have led to good protection against vaginal challenge with feline immunodeficiency virus (personal communication). Furthermore, in studies involving vaginal uptake of antigen, specific antibody-secreting cells are found in the iliac lymph nodes (Johansson et al., 1998).

The above studies would suggest that an immunisation route that utilises these local lymph nodes would be the most appropriate for generation of an optimal immune response in the reproductive tract. However, the local response obtained after direct placement of an immunogen in the vagina has been generally disappointing (Alexander and Bialy, 1994; Gallichan and Rosenthal, 1995) with large doses of antigen and adjuvants often being required to generate a significant response (Thaper et al., 1990). In fact, comparative studies have shown that antibody levels in vaginal fluids are low after intravaginal immunisation, even by comparison with systemic immunisation (Parr et al., 1988; Thaper et al., 1990, 1991). Such poor immune responses following vaginal immunisation with non-replicating antigens are probably due to poor penetration of the epithelium and uptake of antigen. The vagina lacks the classical, organised, mucosa-associated lymphoid tissue (MALT) structures seen in other regions of the mucosal immune system and although MHC class II positive macrophages and Langerhans' type cells are present below the epithelium, King et al., (1998) could find no evidence for these cells migrating to the local lymph nodes during experimental infection of the vagina. Furthermore, uptake of antigen by the vagina is complicated by hormonal factors; proteins can only cross the epithelium during dioestrus, never at oestrus (Parr and Parr, 1990) and antibody responses in the vagina are significantly lower under the influence of oestrogen (Wira and Sandoe, 1987).

Another possible approach is rectal administration of an immunogen, with the premise that it will stimulate immunogenic cells in the lymph nodes that drain both this region and the reproductive tract. However, research efforts to date have not indicated that rectal immunisation is an attractive route; in some studies rectal immunisation did generate specific IgA antibodies in vaginal secretions (Haneberg et al., 1994; Hordnes et al., 1997), but in humans conflicting results have been obtained (Kozlowski et al., 1997; Crowley-Norwick et al., 1997).

As stated above, the female reproductive tract belongs to the common mucosal immune system (CMIS), whereby immunisation at one mucosal site leads to the presence of antibody-secreting cells at a distant site (Mestecky, 1987; McGhee et al., 1993). Hence, immunisation via the oral or nasal route should lead to an immune response in the reproductive tract. A number of studies have revealed that of these two routes, intranasal immunisation yields by far the best antibody response in reproductive fluids; significantly better than those achieved by oral, vaginal, rectal or indeed systemic immunisation. The preferential link between nasal-associated lymphoid tissue (NALT), as compared to gut-associated lymphoid tissue (GALT), and the female reproductive tract, can in part be explained by the homing determinants for primed B cells. It has been shown that the homing receptors which are important for cells to enter the intestinal lamina propria are not shared by the airways (Brandtzaeg, 1997) or the female reproductive tract (Briskin et al., 1997). Similarly, migration of B cells induced in NALT or bronchus-associated lymphoid tissue (BALT), to the gut lamina propria, is negligible (Brandtzaeg, 1992; Brandtzaeg and Halstensen, 1992). However, it has been suggested that common homing determinants may be utilised by NALT and the female genital tract (Brandtzaeg, 1997), which may explain the particularly high levels of specific IgA and IgG in cervico-vaginal secretions after nasal immunisation with a number of different immunogens in mice (Gallichan and Rosenthal, 1995, 1996; Staats et al., 1996; Hordnes et al., 1997; Johansson et al., 1998) and monkeys (Russell et al., 1996). In addition, the nasal route has been found to be efficient at stimulating systemic immune responses and, as discussed previously, serum-derived antibodies do contribute to female genital secretions.

Immune function within the fallopian tubes, uterus, cervix and vagina are distinct, as is evident from the distribution of immune cells within the female reproductive tract. Hence, when determining the most effective immunisation route for immunocontraceptive studies, it will be necessary to analyse the antibody levels at the sites deemed most appropriate for inhibiting sperm function. For example, in the human, the endocervix is by far the most well endowed with antibody-secreting cells and contains lymphoid aggregates most analogous to Peyer's patches in the gut. Never-

theless, the fallopian tube may be a more appropriate site to target sperm-specific antibodies for a number of reasons.

Firstly, the oviduct of most mammals is the site where spermatozoa first encounter the oocyte and where the process of fertilisation is initiated. It is therefore an appropriate site for immunocontraceptive intervention aimed at interfering with sperm–egg interactions. Moreover, there are considerably fewer spermatozoa in the oviduct at fertilisation compared to lower regions of the reproductive tract and hence the opportunity to bind specific antibodies to all sperm present will be greatly increased.

Secondly, in the human and the mouse, numerous antibody-secreting cells are present in the fallopian tubes (Kutteh et al., 1988, 1990; Parr & Parr, 1994) and secretory component is produced by epithelial cells lining the fallopian tubes, indicating both local antibody production and transfer of pIgA to luminal secretions. Furthermore, a significant proportion of the IgG present in oviductal fluids is derived from blood (Oliphant et al., 1978; Lesse, 1988). Indeed, it has been shown that systemic immunisation with sperm-specific antigens in macaques and mice (Kille and Goldberg, 1979; Kurth et al., 1997; Lea et al., 1998a,b), or non-gamete antigens in a variety of species (Oliphant et al., 1977; Yang et al., 1983), results in significant levels of predominantly IgG antibodies in oviductal fluids (up to 27% of those found in serum) which are derived from serum. Hence, enhanced levels of antibodies should be achieved if both local antibody production and transudation from serum can be elicited. Unfortunately, no studies using immunisation routes which stimulate local immune responses in the female reproductive tract have measured the levels of locally-produced antibodies in oviductal fluids.

Finally, several of the sperm-specific proteins that have been proposed as potential target antigens for immunocontraceptive studies (e.g. cyritestin/tMDC I, SP10, SP17) are not exposed on the sperm surface until during the acrosome reaction. Hence, to be effective as contraceptive immunogens, such proteins must be exposed to specific antibodies concentrated in the oviduct at sufficiently high levels during the brief period when these antigens become exposed. Concerns that antibodies may not be accessible to spermatozoa once they have bound to the zona pellucida appear to be unfounded as it has been clearly shown that immunoglobulins pass easily through this matrix (Sellens and Jenkinson, 1975; O'Rand, 1977).

Whilst the oviduct is an obvious target tissue for immunocontraceptive strategies based on sperm-specific antigens essential for gamete interactions at fertilisation, an alternative approach may be to generate antibodies against abundant sperm-specific antigens. This may then lead to sperm agglutination, thereby preventing their progression through the female tract, as has been shown with antibodies raised against human sperm agglutina-

tion antigen-1 (SAGA-1; Diekman et al., 1997) and for several rabbit sperm agglutinating antibodies (Castle et al., 1997). Indeed, the partial contraceptive effect obtained in baboons and foxes following immunisation with the testis-specific lactate dehydrogenase isoenzyme (LDH-C4), has been attributed to antibody-induced agglutination of sperm (Goldberg and Shelton, 1986; Bradley et al., 1996). For such antibodies to be effective in vivo, generation of high antibody levels within the endocervix may be an appropriate strategy, based on the knowledge that naturally-occurring anti-sperm antibodies in semen and cervical mucus are known to agglutinate sperm and prevent their progression through the tract. With this in mind, an alternative approach taken by several groups has been to screen sperm membrane preparations (Naz et al., 1995; Suri et al., 1996) or testicular cDNA expression libraries (Liang et al., 1994) with naturally-occurring anti-sperm antibodies in an attempt to identify potential targets for immunocontraceptive studies.

8. Vaccine delivery systems

Many protein or peptide antigens produced by recombinant DNA techniques or chemical synthesis are only weakly immunogenic when compared to their native forms; hence they commonly require the concomitant administration of immunostimulators in order to elicit an efficient immune response, particularly at a mucosal surface. One adjuvant that has proved successful at enhancing the immunogenicity of vaccines administered both orally and nasally is cholera toxin (CT), a protein composed of A and B subunits, produced by *Vibrio cholerae*. CT has been widely used for studies of mucosal immunity in the intestinal and respiratory tracts, since it strongly induces the production of antigen-specific mucosal CD4 + TH2 cells and secretory IgA responses, as well as inducing IgA and IgG responses in serum (reviewed in McGhee et al., 1994). Moreover, CT is a powerful mucosal adjuvant for co-administered, unrelated antigens. Because the CT-A/CT-B complex can cause undesirable side effects in humans, recent efforts have concentrated on the use of the B subunit alone. However, when administered orally, the use of CT-B as an adjuvant has met with mixed success (Dertzbaugh and Elson, 1991). For example, in one report, orally-administered mixtures of CT-B and antigen were unable to stimulate mucosal immunity unless a very small amount of the holotoxin was added (Lycke and Holmgren, 1986). CT-B does, however, appear to be an effective adjuvant when used for intranasal immunisation, stimulating the production of specific antibodies at a number of mucosal surfaces including the vagina; the mucosal immune responses generated being signifi-

cantly stronger than those following oral administration (Wu and Russel, 1993; Johansson et al., 1998).

Recently, it has been shown that the outer membrane vesicles (OMV's) prepared from group B meningococci possess the necessary properties for inducing efficient mucosal antibody responses, offering an alternative adjuvant to CT. In fact, following intranasal administration, the response to OMV's alone was as strong as that in the presence of CT (Dalseg et al., 1995). Similarly, intranasal administration of whole, heat-killed group B streptococci and *Bordetella pertussis* induced strong mucosal, as well as systemic, antibody responses (Brandtzaeg, 1997), suggesting that surface components of several different bacteria may be suitable as adjuvants for nasal vaccines.

Live vaccine vectors offer another method of stimulating mucosal immune responses. A virulent, recombinant *Salmonella* has been used as a vaccine delivery vehicle and has been shown to stimulate humoral and cell-mediated immune responses (Brown et al., 1987; Maskell et al., 1987; Curtiss et al., 1989; Clemets and Cardenas, 1990). Moreover, oral administration of attenuated *Salmonella* can induce serum- and secretory-immune responses to *Salmonella*-expressed antigens (Katz et al., 1987; Curtiss et al., 1989; Doggett et al., 1993). A number of recent studies have utilised this technology to generate antibodies in vaginal secretions against gamete-specific antigens (Srinivasan et al., 1995; Zhang et al., 1997). Recombinant DNA techniques are being increasingly used to produce chimaeric viruses for use as vaccines; a delivery system which may also be applicable to immunocontraceptive antigens. Oral immunisation with recombinant adenovirus expressing HSV glycoprotein B protects mice from lethal challenge with HSV-2 (McDermott et al., 1980). Similarly, it has recently been shown that intranasal immunisation with recombinant adenovirus expressing HSV glycoprotein B also induces specific antibodies in serum, as well as in vaginal washes of mice (Gallichan and Rosenthal, 1995; Rosenthal and Gallichan, 1997), and provides long term protection from lethal challenge with HSV-2 (Rosenthal and Gallichan, 1997; Gallichan and Rosenthal, 1998).

Other recombinant viruses that have been considered as vectors for the delivery of vaccine antigens include poliovirus, poxviruses, herpes viruses and cytomegaloviruses.

For the control of pest species, such as the rabbit and European red fox in Australia, the use of recombinant viruses permissive to these species (e.g. myxoma and rabies virus) have been considered for the delivery of vaccine antigens (see Boyle, 1994; Tyndale-Biscoe, 1994). However the immunogenicity of the vaccine and host range of the vector must be carefully considered.

Finally, it has been shown that locally-expressed, recombinant vector-encoded cytokines, such as interleukin-5 and interleukin-6, can enhance specific antibody reactivity when co-expressed with vaccine antigens (Ramsey et al., 1994).

Most strategies of vaccination (Hanes and Langer, 1996) require immunopotential to induce efficient immune responses, particularly when using chemically synthesised or recombinant proteins. However, the number of adjuvants available for use in humans is very restricted and the development of new adjuvants is limited by safety considerations. To overcome such limitations, much research has focused on the development of antigen delivery systems that do not require concomitant administration of adjuvant. One such system that has been studied for a number of years is the use of liposomally-encapsulated antigen for the induction of mucosal immune responses, with the formulation of the liposome influencing the nature of the immune response to the incorporated antigen (Nair et al., 1992). Both oral and nasal administration of liposomally-encapsulated antigen elicit strong mucosal immune responses (Michalek et al., 1989; Fujii et al., 1993; Brownlie et al., 1993) and liposomally-incorporated ricin toxoid, or the A chain of ricin, protects mice from lethal challenge for a significant period following vaccination (Griffiths et al., 1997, 1998).

More recently, much attention has been directed towards the use of microspheres of biodegradable polymer, which enable the controlled release of antigens. The microspheres are usually composed of poly-(lactic/glycolic) acid, with the size (Tabata et al., 1996) and formulation (slow versus fast release; Coombes et al., 1996; Gupta et al., 1998) determining the type of immune response elicited. Trials to date have shown that a single inoculation with microsphere-encapsulated antigen leads to strong, long term immune responses that are similar or superior to those elicited by conventional adjuvants (Ertl et al., 1996; Allaoui-Attarki et al., 1998; Gupta et al., 1998; Walker et al., 1998). Moreover, intranasal administration of ricin toxoid encapsulated in poly (lactide-co-glycolide) microspheres provides protection against lethal ricin challenge in 100% of mice, one year post-immunisation (Yan et al., 1996).

9. DNA vaccines

The finding that naked DNA can be taken up and efficiently expressed by cells, when injected into mammalian tissues, has opened up an exciting new approach to vaccine development. DNA is typically administered in the form of a recombinant plasmid with antigen expression driven by a strong promoter and enhancer. When taken up by cells, the DNA is not normally

integrated into the genome, but instead persists as transcriptionally-active, extrachromosomal nuclear episomes. Such DNA vaccines have been shown to elicit both humoral and cell-mediated immune responses (Wahren, 1996; Montgomery et al., 1997), qualitatively similar to live attenuated vaccines, but without the inherent safety hazards of infectious agents.

There are two general approaches to DNA immunisation; saline injection and 'gene gun' delivery. Saline injections deliver DNA into extracellular spaces, 'gene gun' delivery systems bombard DNA-coated gold particles directly into cells through cell membranes. However, these two different methods of DNA delivery appear to elicit different types of immune responses; predominantly initiating either type 1 (TH1) or type 2 (TH2) T cell responses (Barry and Johnston, 1997; Robinson and Torres, 1997). 'Gene gun'-delivered DNA initiates responses by transfected or antigen-bearing Langerhans' cells that move in lymph from the site of skin bombardment to the draining lymph nodes (Robinson and Torres, 1997). In contrast, following intramuscular injection, free DNA appears to move via the blood to the spleen, where professional antigen-presenting cells initiate responses. However, the differing responses achieved by these modes of delivery may in part be due to differences in the amount of DNA administered; 100-fold less DNA being required for 'gene gun' inoculation (Barry and Johnston, 1997). The potential of DNA vaccine technology for immunocontraception is suggested by the recent demonstration that significant antibody levels can be obtained in vaginal secretions following local inoculation with a model, DNA-based antigen, delivered by 'gene gun' technology (Livingston et al., 1998).

10. Conclusion

Despite many attempts over the past decade, the potential of immunocontraception as an effective approach to birth control has yet to be convincingly demonstrated. Much of the work to date, based mainly on rodent model systems, has focused on gamete-specific antigens implicated in sperm–egg binding and fertilisation as the immunocontraceptive target. However, the results of such studies have been largely disappointing. We attribute this to two fundamental flaws in the way these preliminary trials have been designed and carried out; choice of antigen and choice of immunisation route. We have argued in this review that current evidence points towards a number of gamete-specific antigens actively participating in sperm–egg interactions and that these antigens may act co-operatively. Hence, no single antigen may be indispensable and therefore immunisation with a single antigen, whilst possibly reducing fertility, is unlikely to lead to

infertility. Instead, an effective immunocontraceptive may need to be based on a cocktail of gamete-specific antigens. We have also argued, in the light of available data, that systemic immunisation is not an appropriate, effective or reliable means of eliciting an optimal immune response in the female reproductive tract. Alternative routes of immunisation, which are likely to promote mucosal immune responses in the reproductive tract, offer what should be a more valid approach. Indeed, the suggestion that NALT and the female genital tract may utilise common homing determinants would indicate that nasal immunisation may warrant particular consideration as an effective and acceptable approach to human immunocontraception in the future.

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