A recent paper by Lachapelle et al. (1996) described differences in follicular fluid leukocytes from patients with unexplained infertility and endometriosis compared with tubal factor. Patients with unexplained infertility had a higher proportion of T cells and with endometriosis higher natural killer (NK), B lymphocytes and monocytes. We find these observations intriguing since we have previously reported increased concentrations of autoantibodies in follicular fluid of infertile women with unexplained infertility and endometriosis (El-Roeiy et al., 1987). These two observations (El-Roeiy et al., 1987; Lachapelle et al., 1996) led us to reconsider the significance of the role of immune processes in infertility.

It is now well established that infertile women who reach a treatment stage that involves assisted reproductive technology (ART), demonstrate a very high incidence of autoimmune abnormalities (Fisch et al., 1991; Birkenfeld et al., 1994). A similar finding has been reported in women with endometriosis (El-Roeiy et al., 1988; Sher et al., 1994).

Our earlier study demonstrated that infertile women with abnormal peripheral autoantibody concentrations had demonstrated highly elevated autoantibodies within follicular fluid. Interestingly, however, autoantibody abnormalities within follicular fluid involved only antiphospholipid specificities and not antihistones or antipolynucleotides, even if those were fertilization and naturally occurring antibodies: evidence for increased interest in the ‘immunology of the ovary’ is required. In addition, however, maybe more attention should be given to potential immunological therapies of infertility. Some preliminary data have recently suggested that otherwise unexplained implantation failure can be corrected with various forms of immunotherapy (Coulam et al., 1994; Sher et al., 1994; Dmowski et al., 1995). From an immunologist’s view it is time for well controlled randomized prospective studies to test this hypothesis.

References

Immunological causes of ovarian infertility and repeated implantation failure—two aspects of the same problem?

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As pointed out by Gleicher and Coulam (1997) a number of studies have so far reported that women with various causes of infertility more often than normal women prove positive for a series of autoantibodies (e.g. antiphospholipid, antinuclear and antispermatozoal antibodies) in peripheral blood (Taylor et al., 1989; Sher et al., 1994; Nip et al., 1995). High frequencies of autoantibodies can also be found in follicular fluid of these patients (Nip et al., 1995). Furthermore, the presence of non-organ specific and organ-specific autoantibodies (anti-ovarian and anti-thyroid antibodies) in serum or follicular fluid has been demonstrated to have a negative affect on the success rate in infertile women undergoing in-vitro fertilization (IVF) with embryo transfer (El-Roey et al., 1987; Geva et al., 1996). Investigations of lymphocyte subpopulations in follicular fluid have also shown different distributions of T- and B-lymphocytes and natural killer (NK)-cells depending on the cause of the infertility (Lachapelle et al., 1996). There is thus much evidence that systemic or local immunological disturbances in the ovary are associated with some kinds of female infertility and repeated IVF failure.

When dealing with immunological disturbances in the ovary, we are in the rare and pleasant condition that local immunological factors in the organ of interest can be easily studied due to the availability of follicular fluid aspirated as a part of the IVF procedure.

Interesting discoveries have already been made by investigating follicular fluid but some questions remain unanswered: what would be the T-helper type 1/T-helper type 2 (Th1/Th2) phenotype of follicular-fluid mononuclear cells from different categories of infertile women (and also fertile women) when investigated by flow cytometry? Which cytokines do these cells produce when exposed in vitro to relevant antigens (e.g. proteins from zona pellucida, spermatozoa and trophoblast)? Do they react with a so-called Th1 response or a Th2 response? These are interesting questions since many organ-specific diseases (Bach, 1995) and probably also recurrent miscarriage are characterized by a Th1 rather than a Th2 response to target antigens (Hill et al., 1995). It is also important to characterize the antigens which are the targets of the autoimmune processes in the ovary.

Gleicher and Coulam (1997) call for more research on the immunology of the ovary and, at the same time, call for trials of immunotherapy e.g. in patients with repeated IVF failures. I believe it is important to keep in mind that the potential target for immunotherapy in repeated IVF failure is not the ovary but the endometrium.

Whereas investigations of immunological disturbances in the ovary and follicular fluid are interesting in elucidating causes of unexplained infertility, it is difficult to see how these disturbances can affect the pregnancy rates achieved after IVF/embryo transfer. Most studies have reported that the number of eggs retrieved per cycle, the number of fertilized eggs and the number of embryos transferred per cycle are similar in women with or without autoantibodies or embryotoxic factors in serum (El-Roey et al., 1987; Dokras et al., 1993; Dmowski et al., 1995; Geva et al., 1996). It seems to be the implantation rate of the embryos which is negatively affected by the presence of autoimmune disturbances in serum and follicular fluid. Immunological disturbances in follicular fluid might reflect similar changes in the endometrium.

Now, 20 years after the beginning of the era of assisted human reproduction, the implantation rate in IVF/embryo transfer is still only 12–15% per embryo transferred, despite a rapid improvement of ultrasound, protocols for ovarian stimulation and embryo culture techniques. Low implantation rates are the primary reason for the high cost of IVF and related procedures to infertility patients (Edwards, 1995).

A series of studies have suggested that immunological factors are involved in the implantation processes in animals and in humans. NK-like cells positive for the CD56 marker are sparse in the endometrium in the proliferative phase of the menstrual cycle but increase significantly in number in the luteal phase and in the early part of pregnancy (King and Loke, 1991). These cells may play a role in the implantation events and control the extent of subsequent trophoblast invasion (Loke and King, 1996). CD56 positive cells produce a series of cytokines which affect trophoblast growth and the CD56 positive cells are themselves under strict regulation by cytokines (Loke et al., 1995). In mice it has been demonstrated that the cytokine leukaemia inhibiting factor (LIF) is crucial for implantation of embryos (Stewart et al., 1992). Biopsies of endometrial tissue from women with repeated IVF failure due to deficient implantation have been shown to produce less LIF in vitro than do endometrial biopsies from fertile women (Chauvat et al., 1995). There is also much evidence that interleukin-1 (IL-1) in the endometrium plays an important role for implantation in mice and humans (Simón et al., 1994). Furthermore, preimplantation embryos produce IL-1. High IL-1 production by embryos has been reported to be associated with a high implantation rate after embryo transfer (Sher et al., 1991).

There is thus substantial evidence that the endometrial cells and the embryo which is being implanted ‘crosstalk’ via a series of cytokines and this, in a complex manner, regulates the implantation process. Errors in this crosstalk due to insufficient or unbalanced production of cytokines or inadequate expression of cytokine receptors is suggested to decrease the possibility of implantation.

How do these theories cope with the finding that a series of autoantibodies in the ovary or serum seem to be associated with unsuccessful implantation of transferred embryos? Is there one population of patients with implantation failure caused by autoantibodies and another in which failed implantation is caused by imbalances in cytokine interactions between...
embryo and decidual cells? In autoimmune diseases, autoantibodies are rarely the pathogenetic factor; the tissue lesions characterizing these diseases are frequently caused by cellular mechanisms. There is of course the possibility that the autoantibodies themselves cause failed implantation; antiphosphatidylerine antibodies have been suggested to inhibit fusion of cytotrophoblast cells to form a syncytiotrophoblast (Rote et al., 1992). However, the broad autoantibody response against both organ-specific and non-organ-specific targets which characterizes these patients (El-Roeiy et al., 1987; Nip et al. 1995; Geva et al., 1995, 1996), rather than the specific response against phosphatidylserine expected if antibodies against this phospholipid happened to be the pathogenetic factor, supports the theory that more complex immunological disturbances underlie repeated implantation failure. The autoantibody formation might be a consequence of repeated excessive levels of oestrogen or follicle punctures in these patients (Moncayo et al., 1990; Barbarino-Monnier et al., 1991). Alternatively, it may be an epiphenomenon since an imbalance of cytokine production (in the actual case in the endometrium) and autoantibody formation is often associated as seen in the case of Th1 cytokine responses and organ-specific autoimmune disease (Bach, 1995). Excessive production of certain cytokines by mononuclear cells is under the control of human leukocyte antigen (HLA-DR) alleles which are also associated with the formation of several autoantibodies (Pociot et al., 1993). If successful implantation is dependent on well-functioning cytokine interactions between endometrial mononuclear cells and embryonic cells, then various forms of immunotherapy could theoretically increase pregnancy success rates after IVF/embryo transfer. Uncontrolled trials have reported promising results with respect to prednisone (Dmowski et al., 1995), heparin and aspirin (Sherr et al., 1994) and intravenous immunoglobulin (Coulam et al., 1994). These potential treatments should be tested in prospective, placebo-controlled trials in women with unexplained infertility attempting spontaneous conception and also in women with repeated IVF failure.

In recurrent miscarriage, placebo-controlled trials of immunotherapy have been few and small, primarily due to the relative rarity of the diagnosis. Since ~40% of all women undergoing IVF/embryo transfer fail to have a child after three embryo transfer attempts, the opportunity to assemble an adequate number of patients for such trials seems better. However, it must be kept in mind that an eventual effect of immunotherapy in infertile women attempting conception without the use of IVF/embryo transfer and related techniques can be attributed to an improvement of immunological interactions both in the ovary and the endometrium, whereas an effect of immunotherapy in women undergoing IVF/embryo transfer can probably only be attributed to an improvement of immunological conditions in the endometrium.

References