

DISCUSSION

During the last decade, much evidence has accumulated supporting the idea that unsuccessful pregnancies may be due to aberrations of the immunologic process that are part of the normal pregnancy. Medawar (1953) suggested that the foetus is not rejected as a foreign graft possibly because of the following reasons : i) the conceptus is not immunogenic and therefore does not evoke an immunological response ii) pregnancy alters the maternal immunological responses iii) the uterus is an immunologically privileged site and iv) the placenta is an effective immunological barrier between the mother and the, as yet, immunologically incompetent foetus.

Since this hypothesis was proposed 38 years back, at recent years many workers have shown that the conceptus is immunogenic and that it does evoke a response from the mother (Kirby, 1969 ; Raghupathy & Talwar, 1983 ; Cauchi et al., 1987). It is an alteration of this response that causes abortion (Johnson et al., 1985). Several groups have shown that it is normal for the mother to make an immunological response to the foetus (Tongio, Berrebi and Mayer, 1972 ; Mowbray et al., 1983 ; Beer and Billingham, 1977) and that the mother

when pregnant, can respond to a wide variety of immunological stimuli (Gill & Repetti, 1979). Singal (1984) demonstrated that the normal pregnant mother contains blocking antibody in her serum which is absent in habitual aborters. These antibodies have been shown to inhibit the cell-mediated immune response and may induce suppressor cells, which are probably important for defense mechanisms against rejection by mothers. But these studies could not provide much information regarding the correlation between the presence of HLA alloantibodies and the cell mediated immune response of the mother.

The objective of the present study was to find out the role of HLA alloantibodies during pregnancy as well as to correlate the cell mediated immune response with the incidence of HLA aloantibodies in normal and unsuccessful pregnancy.

In the present study, to assess the immune response of the women during pregnancy including the presence of HLA alloantibodies, several parameters have been investigated like HLA haplotype and gene frequencies, quantitation of serum immunoglobulins, mitogenic stimulation of lymphocytes in in vitro etc.

A good number of papers have been published during the last decade to explain the histocompatibility/incompability between the foetus and the normal or unsuccessful pregnant

mothers. Few workers have suggested that the foeto-maternal histocompatibility antigens does not influence pregnancy characteristics predispose to abortion (Jazwinska et al., 1987; Kilpatrick et al., 1989).

The association of specific HLA antigens in case of normal or unsuccessful pregnancy and the mother in case of unsuccessful pregnancy is still controversial though several workers have supported these views (Reznikoff-Etiavent et al., 1987). Christiansen et al., (1989) have shown that the tendency to unexplained RSA (recurrent spontaneous abortion) in women seems to be associated with a gene or gene region in or near the HLA region. These genes themselves are malfunctioning, causing a defective immune response directed from the mother against the semi-allogenic embryo or from the embryo against the mother resulting in miscarriage (Wegmann, 1987). Smeraldi (1985) demonstrated that no significant increase of HLA sharing in the RSA couples over that in the normally fertile couples. This confirms the findings of several other workers (Caudle et al., 1983 ; Oksenberg et al., 1983), which is contradictory to the hypothesis that the sharing of HLA antigens is associated with post-fertilization pregnancy wastage in humans (Beer et al., 1981 ; McIntype and Faulk, 1983 ; Gerencer and Kastelan, 1983 ; Unander and Olding, 1983 ; Reznikoff-Etiavent ^aet al., 1984).

In the present study we observed that the frequency of few HLA antigens were either higher or lower in case of unsuccessful pregnancy. The frequencies of the antigens A1, B5, B15, Cw1, Cw5 of class I and DR1, DR2, Dqw1 of class II were significantly higher where as antigens Cw1, Cw2 of class I and DQw2, DQw3 of class II were significantly lower in case of unsuccessful pregnancy. We also observed few haplotypes were with significant positive linkage disequilibria in case of unsuccessful pregnancy.

On the basis of the earlier works and also from the present observation it may be said that either there are some genes linked to the HLA region or the sharing of homologous antigens and the presence of few HLA haplotypes with strong linkage disequilibria possibly increase the susceptibility of the early rejection of the foetus in case of unsuccessful pregnancy. Different results including ours did not indicate the associations of common antigens or the association of common haplotypes or even the sharing of the same homologous antigens in all unsuccessful pregnancies. It is possibly because of the presence of different HLA antigen frequencies in different population of the world according to the geographic distribution or migration.

The acceptance of the foetus by the mother is the only physiological instance in which a semi-allogenic allograft

is normally not associated with the rejection. To date, the factors leading to the materno-foetal tolerance remain unknown, but several lines of evidence suggest that genes of the major histocompatibility complex (MHC) might play an important role either directly or indirectly. The high frequency of maternal anti-HLA antibodies reported in women with successful pregnancies (Power et al., 1983 ; Fizet and Bousquet, 1983 ; McIntyre et al., 1986) and low frequency in women with repetitive spontaneous abortion (Johnson et al., 1985) suggest that HLA alloimmunization can in some way enhance the pregnancy. There is strong evidence that anti-HLA alloantibodies are probably involved in the successful outcome of pregnancy (Beer et al., 1981).

In the present investigation we observed that the incidence of HLA alloantibodies against class-I antigens were higher in normal than unsuccessful pregnant women but HLA alloantibodies against the class - II antigens were found lower in normal pregnant women compared to that of the unsuccessful pregnancies.

The absence of HLA alloantibodies in case of unsuccessful pregnant women indicate the possible sharing of homologous HLA antigens between the foetus and the mother. We know that in case of organ transplantation at least 50% HLA matched organ in some cases and 100% HLA matched in other cases are

necessary for the survival of the graft, but it is interesting to note that this fact is reverse in case of pregnancy. The foetus is a semiallogenic graft and is rejected by all mothers even in case of normal pregnancy. The difference between the normal and the unsuccessful pregnancy is that in case of normal pregnancy, the rejection is delayed upto approximately 280 days whereas it is early to very early in case of unsuccessful pregnancy. As it is indicated from the different works and also from our observation that the presence of HLA allo-antibodies (especially against class-I antigens) are really necessary for the survival of the foetus, it seems that the HLA allo-antibodies have some role to mask or to cause the inhibition of the CMI response of the mothers, which actually prevent the graft rejection.

The presence of higher percentage of HLA alloantibodies against the class-II antigens in case of unsuccessful pregnancy remain unexplained and need further investigation.

In the present study the concentration of IgG level was found higher in normal than the unsuccessful pregnancy and the concentration of IgM was lower in normal when compared to that of the unsuccessful pregnancy. The high level of IgM is indicating the strong primary immune responsiveness of the mother in case of unsuccessful pregnancy.

The high level of IgG and higher incidence of HLA alloantibodies (against class I antigens) in normal pregnant compared to the unsuccessful pregnant women may have some role in protecting the foetus separately or with the combination of both (Unander and Olding, 1983 ; Beer et al., 1981 ; McIntre and Faulk, 1983 ; Gerencer and Kastelan, 1983).

As it is known that the cell mediated immunity is mainly responsible for the HVG reaction, in the present study we also investigated the degree of the cell mediated immune response of the normal and unsuccessful pregnant women through several parameters like the in vitro blastogenesis of the lymphocytes, the ratio of T-cell subsets etc.

Examination of the course of lymphocyte sub-populations during pregnancy has attracted considerable interest in recent years, and had led to some what contradictory results. Few workers (Baines et al., 1977 ; Dodson et al., 1977) found no changes in T-lymphocytes, B-lymphocytes or macrophages in normal pregnant women compared to the unsuccessful pregnant women. Bulmer et al., (1977) found a decreasing T- lymphocyte count during normal pregnancy together with an increasing B-lymphocyte count when compared with the unsuccessful pregnant women.

Blastogenesis is one of the indicator of activation of lymphocytes. The initial interaction between the lymphocytes

and mitogen is the binding of the mitogen to the receptor on the lymphocyte surface. Normally the lymphocytes after getting stimulation became larger in size and form blast cells and undergo differentiation to become an effector cell. There are many changes in the metabolic activities of lymphocytes between the initial stimulation by mitogen and the start of DNA synthesis.

In the present study we observed that the total percentage of blasts as well as the T and B-blast cells were higher in case of unsuccessful than that of the normal pregnant women. So it may be said that the complications arise in case of unsuccessful pregnancy may be due to the strong immune responsiveness of the mother against the foetus.

The nature of this mechanism is not clearly understood but possibly a single factor is involved which may be common both in the cell mediated and the antibody mediated immune response.

The high percentage of B-blast cells in case of unsuccessful pregnant women may be due to the stimulation of the B-cells by the factor secreted by activated T-cells or may be due to the antigenicity of the trophoblastic cells and in that case the plasma cells (B-blast cells) will secrete antibodies against the trophoblastic cells. Possibly HLA

alloantibodies help to block the trophoblastic antigen as well as may inhibit the function of the Th- cell.

It is generally accepted that the primary mechanism for the destruction of allografted tissue is through the action of a class of thymus-dependent or T-lymphocytes. Within any individual, many distinct clones of T-lymphocytes exist with surface structures or T cell receptor for antigen, specific for a very narrow range of antigenic determinants. Conversely, in the total population of species, such as humans, a complex array of distinct antigenic patterns exist in any tissue type. Thus, each individual has several distinct genetically determined patterns on all eukaryotic body cells with the MHC proteins as most important antigenic system. If the MHC pattern of the donor tissue does not closely match the pattern of the host, the tissue is recognised as foreign by the immune system and those specific classes of T-cells capable for reacting with it mount an immunogenic response. It is this genetic determination of tissue antigen patterns that explains why transplants between identical twins are successful, between close relatives less frequently so, and between unrelated individuals virtually impossible without some means of immune suppression.

Two general mechanisms are responsible for tissue rejection. In the first, allogenic tissue with surface class I MHC antigens interact with cytotoxic CD8⁺ T-cells, which are

activated by interleukin 2(IL-2) to produce agents toxic for these target cells (the donor tissue). In the second process, allogenic class II MHC antigens interact with helper $CD4^+$ -bearing T-cells which then recognise the foreign tissue as 'presenting cells' and trigger the activation of antiallogenic B-cells to release antibody specific for the donor tissue. Other non-specific phagocytic cells are then called in by these helper T-cells to cooperate in the process of destroying the foreign tissue. The second helper T-cell response, however, is the most important in the rejection of foreign tissue as patients with matched class II antigens display much better allograft tolerance than those matched for class I antigens.

We observed higher percentage of Ts-cells in normal pregnant women compared to the unsuccessful pregnant women. The foetus, functions as an allograft with dissimilar HLA haplotypes, is tolerated by the mother upto some period (approx,280 days) due to immunological suppression.

In the present investigation we also observed that the percentage of $CD8^+$ T-cells was much higher in case of non-pregnant (virgin) women. But the ratio of $CD4^+$ and $CD8^+$ T-cells was lower in normal pregnant than the unsuccessful pregnant women, indicating more pronounced suppressor activity in case of normal pregnant women. Possibly the increased function of the suppressor T-cell is co-responsible for maintaining the mother's tolerance of the paternal allograft, the foetus.

So far it has been discussed through several parameters that during normal pregnancy the immune response of the mother is suppressed to some extent and same is absent in case of unsuccessful pregnancy. The higher ratio of $CD4^+$ and $CD8^+$ T-cells in case of the unsuccessful pregnancy though partially proves the above idea but in the present study we also investigated the blastogenic transformation of the lymphocytes in in vitro with the mitogenic stimulation to see whether the sera of the successful pregnant women contain any factor(s) to block the surface receptor of the T-cells and thereby inhibit the CMI response.

The lymphocytes of the normal and unsuccessful pregnant women were cultured separately by using autologous sera for different hours and observed that in case of unsuccessful pregnancy, the percentage of blast cells was much higher than the normal pregnancy. This may be due to some factor(s) those are present in the serum and possibly responsible for the inhibition of cell activation. This was evidenced when the lymphocytes of the unsuccessful pregnant women were cultured with the sera taken from the normal pregnant women having same blood groups and compared with the degree of blastogenic transformation of the lymphocytes of normal pregnant women, cultured with autologous serum. In both the cases the percentages of blast cells were more or less same and lower than the percentage of blast cells of unsuccessful pregnant women, cultured with autologous serum.

As it is indicated from the previous discussion that the CMI response is slightly inhibited during the pregnancy, especially in case of normal pregnancy possibly to avoid the rejection of the foetal allograft, it has been investigated whether the degree of the blastogenic transformation of lymphocytes of normal pregnant women just before delivery and after delivery were comparable with degree of the blastogenic transformation of the lymphocytes of non pregnant women (virgin). The lymphocytes of non pregnant (virgin), immediately before delivery and after delivery of normal pregnant women were cultured with autologous sera. The blastogenic transformation of the lymphocytes of non pregnant (virgin) women and delivered women were very close to each other and higher than the pregnant women before delivery.

From the above results it proves that during pregnancy the immune response, especially the CMI response, is inhibited till delivery in case of normal pregnant women and if the inhibition is not upto the level to avoid graft rejection, the foetus will be rejected as it happens in case of unsuccessful pregnancy. Antibody mediated immune response follows a different path where we have seen stronger primary immune responsiveness in case of unsuccessful pregnancies. It seems that the CMI is regained slowly after delivery. Further works are need to be done in this direction to prove the exact role of IgG , IgM, Ts & Th during pregnancy. The level of IgG & IgM is need to be

compared at different time intervals with non-pregnant, pregnant of different trimesters and after delivery as well as to know the exact role of suppressor and cytotoxic T-cells ($CD8^+$) separately, experiments are need to be done by eliminating the suppressor cells from the $CD8^+$ T-cells population.

In conclusion, the present investigation has focussed the role of the HLA alloantibodies which could inhibit the cell mediated immune response directly or indirectly by immunomodulation to provide the suppression of the immune system of the mother for avoiding the foetal rejection. The incidence of the higher percentage of HLA alloantibodies against the HLA class II antigens in case of unsuccessful pregnancy may also be responsible for the foetal rejection. High level of IgM and higher percentage of blast cells as well as the higher ratio of $CD4^+$ and $CD8^+$ T- cell in case of unsuccessful pregnancy are clearly indicating the strong immune responsiveness (may be primary) of the unsuccessful mother. The unsuccessful pregnant women may become successful possibly by increasing $CD8^+$ T-cells or by eliminating the $CD4^+$ T-cells upto the desired ratio or by immunosuppression.