Genetic factors in human reproduction a trade off between procreation and longevity

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General discussion

GENERAL DISCUSSION

1. GENETIC FACTORS AND HUMAN REPRODUCTION

The two genetic factors highlighted in this thesis are considered to interfere with human reproduction through different pathways. The first pathway studied is the innate immune response by way of cytokines that acts through regulation of an inflammatory process. The process of inflammation is known to induce angiogenesis[1]. Angiogenesis is the formation of new blood vessels from pre-existing ones, and is acknowledged to play an essential role in the process of embryo implantation[2;3] and cytokines are considered to be involved in this process[4]. The cytokine interleukin-10 (IL10) is studied in detail in this thesis as it is thought to have an important role in pregnancy[5;6]. IL10 tempers pro-inflammatory cytokines and induces a shift towards a more anti-inflammatory immune response, creating a favourable balance for the acceptance of the semi-allogenic embryo in the maternal uterus[6].

The second pathway studied is by way of increased general coagulability due to the factor V Leiden mutation. Coagulation is a necessary step during the implantation process considering that the blastocyst invades the trophoblast with numerous capillaries[2]. If excess bleeding would occur the fate of the embryo may be jeopardised. In this process the factor V Leiden status of the mother and the embryo are separate entities. Possibly the Factor V Leiden mutation in an embryo alters the chance of implantation during the very early phases of life.

2. TRADE OFF?

It is questioned whether the molecular mechanisms that are described in this thesis would support the disposable soma theory; i.e. would fit the trade off between reproductive success and longevity. Regulation of immunity is an obvious candidate because the adverse conditions in our (natural) habitat necessitate large investments to fight infections and therefore reach (post)reproductive age. Cytokines are critical signalling molecules. Tumor necrosis factor-α (TNFα) for instance will initiate an inflammatory response to fight infection. Regulatory signals from IL10 taper the inflammatory response and prevent collateral damage after the infection has been overcome. It has now been shown that the production capacity of these cytokines appears to be under tight genetic control[7].

In an earlier study the levels of IL10 and TNFα were studied in first-degree relatives of patients who suffered meningococcal disease, an infection that is widely present in Africa and occasionally surfaces in developed countries[8]. It was therefore assumed that families of those patients who had died would have a distinct pattern of cytokine activity. Almost without exception, the level of pro-inflammatory TNFα in all of these cases was low, and the level of the anti-inflammatory IL10 was high. The interpretation of these data was that subjects with an innate propensity towards anti-inflammatory responses are at an increased risk of death from infection. IL10 responsiveness was furthermore found to be reduced at old age. Innate IL10 responsiveness was significantly reduced in subjects over 85 years old compared to both 14-40 year olds and 41-75 year olds. When LPS-induced cytokine levels were compared between a random selected subgroup of 85-year old and younger control donors, the 85-year old study participants produced lower levels IL10 (16). Whether the various IL-10 promoter alleles are of influence on longevity remains inconclusive. An Italian study found that the *IL-10* gene SNP -1082G-A allele had a significant influence on the attainment of longevity in men[9] this in contrast to a Finnish population study where IL-10 promoter alleles and haplotype frequencies were not different between nonagenarians and controls[10].

In contrast with fighting infection, which requires a strong inflammatory host response, reproductive success depends on a tolerant immune response[6], though it is possibly not essential[11]. The effect of IL10 in early pregnancy can be explained in two ways. Firstly, the tissue antigens of an embryo are partly of paternal origin, therefore at the fetal−maternal interface immune reactions must be suppressed to allow pregnancy to be accepted and to proceed. Inhibition of the pro-inflammatory (Th-1) immunity, for instance as a result of the immuno regulation of IL10, is considered necessary for the acceptance of the semi-allogenic fetus[5;12;13]. Secondly, the effect of IL10 may be explained by inhibiting the local inflammatory reaction. This inflammatory reaction has been shown to be important for successful pregnancy in mice[14]. Possibly very high levels of IL10 decrease local inflammation possibly reducing the chance of successful implantation. The limits of optimal IL10 levels are probably regulated. However this may indicate that the effects of differences in genetic dissimilarity in IL10 production will initially have an effect on the immune system rather than on the minute amounts of inflammation necessary to induce a vascular response.

In this thesis we have shown that the cytokine profile of women with impaired fertility (defined as having at least three consecutive spontaneous miscarriages) is markedly different when compared to the profiles of women of normal fecundity[15]. The data on the cytokine profiles help to elucidate two phenomena. First, they can explain why British aristocrats (see introduction), who lived longer, were less likely to have successful pregnancies. Their innate immune system favoured resistance to infection but at the same time prevented pregnancy from proceeding, a trade-off that was even stronger in times when the environmental conditions were relatively poor. Second, it explains why a genotype associated with impaired fertility might have persisted in spite of its obvious disadvantage with regard to evolutionary fitness. Selection for resistance to infection is traded against selection for fertility, resulting in a compromise that is optimal for the fitness of the species in a specific environment.

The molecular mechanisms that can explain the trade off between reproductive success and longevity are not confined to the regulators of immunity. In this thesis the factor V Leiden mutation has been postulated to facilitate the process of embryo implantation. It may be beneficial for an embryo to possess this gene mutation early in life, for instance increasing the chance of implantation, but on the other hand increasing the chance of a possibly life threatening thrombotic event later in life. However, factor V Leiden has been shown to have the same prevalence in old age as early in adult life[16]. Possibly the increased therapeutic options available in prophylaxis and treatment of thrombotic events play a role in the similar prevalence found at high age. Whether a 'trade off' in this sense exists remains unclear.

3. CYTOKINES AND COAGULATION IN PREGNANCY

Inflammation and coagulation are important factors in pregnancy. Normal pregnancy is both an acquired hypercoagulable and inflammatory state[17;18]. A clear association between factor V Leiden and cytokine production (in particular IL10 and TNFα) has not been observed so far[19]. Effects of other cytokines on coagulation and vice versa have been stated in the literature. First, a direct effect of cytokines on coagulation is proposed by up-regulating fibrinogen-like protein 2 (fgl2) prothrombinase[20]. Fgl2 is a glycoprotein that is capable of directly cleaving prothrombin to thrombin leading to fibrin deposition[20]. Increased levels of Th1 cytokines (i.e.

TNFα) have been shown to activate coagulation via up-regulating fgl2 [21]. Fgl2 is thought to play a role in spontaneous miscarriages[22;23].

Second, a pathway is proposed through the presence of circulating pro-coagulant microparticles (cytoplasmatic components and membrane-derived elements from various cells) found in pregnancy[24]. These microparticles also act as potent pro-inflammatory agents[25]. The concentrations of these pro-coagulant microparticles are increased in the peripheral circulation of both women with both early and late miscarriages compared to women with normal pregnancies[25;26].

Third, a possible link that a connection exists between coagulation and inflammation may be that treatment with anticoagulant medications, such as heparin may not only have properties regarding anticoagulation, but also may have other means of interfering with pregnancy[27;28]. Interactions between heparin and cytokines have been published, for instance inhibiting the anti-inflammatory cytokine IL-8 in rats[29] but also inhibiting the inflammatory TNFα in a mouse model[30]. It has furthermore been suggested that heparin interferes with the adhesion of the blastocyst to the endometrial epithelium and the subsequent invasion[31]. Further evidence of this relation between heparin and pregnancy remains to be verified.

4. TH-1/TH-2 PARADIGM IN PREGNANCY?

As been stated previously, pregnancy has been classified as a Th-2 mediated phenomenon where suppression of pro-inflammatory cytokines in the decidua is assumed to be important for successful placentation[5;32]. It has been suggested that this Th-1/Th-2 hypothesis represents an oversimplification of the situation[33;34]. Cytokines are produced not only by T-helper cells (Th-1 or Th-2 cells) but also by cells other than immune cells, including macrophages, epithelial and stromal cells of the endometrium and decidual and cytotrophoblast cells of the placenta[35]. Also early IVF embryos have been found to produce various cytokines including IL10[36]. Moreover, it is acknowledged that cytokines have overlapping functions and can be both anti- as pro-inflammatory[37]. As a result it seems less probable that there is a strict separation between a Th-1 and Th-2 immune response and a more complex core is likely to exist.

However, in principle, the results in this thesis support the original hypothesis that enhanced secretion of anti-inflammatory (Th-2) cytokines is a characteristic of a normal physiologic pregnancy[5]. We found that women with a high fecundability (who were pregnant within 3 months) were 16 times more likely to have a high IL10 and low TNFα responsiveness compared to women who suffered recurrent miscarriages. Also in women with rheumatoid arthritis (a Th-1 mediated disease) who had suffered a miscarriage had higher likelihood of developing a more severe disease measured by a more severe joint damage in the first two years of presenting. At a genetic level only one IL10 SNP was found to be related to fertility: the IL10 -2849 AA genotype increased the likelihood of remaining childless in a female married population and increased the time interval between marriage and birth of the first-born child. The relation between non-exon SNPs and gene function is however difficult to prove by either biochemical or molecular biological methods. This is because it is unknown which stimulus eventually leads to the increased IL10 secretion in pregnancy. Given the fact that little knowledge is present on the exact biological process it is not known which transcriptions factors are involved in addition to whether a change in the transcription factor binding site is relevant. Thus we examined whether IL10 -A2849G was merely a tag of a haplotype or whether it alone was the best predictor of fertility characteristics. Indeed, no relation between the IL10 haplotypes and fertility or fecundity could be found, although it was generated by a limited number of SNPs. We therefore proposed that the IL10 -A2849G SNP is related to an altered gene function in regard to fertility. The low IL10 responsiveness that is particularly found in relation to the IL10 –2849 AA genotype may be crucial in explaining these results[15;38]. A low IL10 responsiveness may reduce the chance of developing a successful pregnancy.

It is beyond doubt that the mechanism stated above is just a small part of a far bigger story. IL10, as a Th-2 cytokine is beneficial for a successful pregnancy. However, not only Th-2 but also a number of Th-1 cytokines seem crucial for successful implantation of the blastocyst. This has been indicated in animal studies. Interestingly, IL10 knock-out mice have been shown to have normal reproduction results[11;39] contrary to general inflammatory knock-out mice that all resulted in implantation failure[40;41]. Maybe we should rephrase Wegmann's hypothesis of pregnancy as a Th-2 phenomenon into 'pregnancy is a Th-2 phenomenon that cannot occur successfully without Th-1 components taking place'.

5. A TOO EASY ACCEPTANCE OF A PREGNANCY?

Theoretically, miscarriages can be seen as a safety net to filter out a chromosomal or morphological abnormal embryo. If miscarriages would not occur, many more severely abnormal children would be born, most probably not surviving birth[42]. In this thesis a reduced number of first trimester miscarriages was found in relation to maternal factor V Leiden carriership, without altering the miscarriage rate overall. An explanation of this finding remains to be elucidated. One suggestion may be that as factor V Leiden increases coagulation, it may well interfere with coagulation locally at the site of implantation of the blastocyst in the endometrium. The blastocyst is known to invade blood vessels as it penetrates into the luminal epithelium of the endometrium forming the trophoblast that contains multiple cavities with maternal blood[43]. It is possible that a decreased likelihood of bleeding may reduce the chance of the early pregnancy to fail (i.e. the blood loss itself as a cause of the pregnancy failure, not as a result). Factor V Leiden might increase the chance of implantation due to the enhanced coagulability, conceivably by decreasing the amount of blood loss occurring at implantation[44]. Possibly, this may increase the chance of a pregnancy to continue regardless of the existence of a chromosomal or morphological abnormality in the embryo. This would assume that the abnormal embryo will miscarry later in pregnancy, in a next step of Mother Nature's safety net. Another theory may be that the high coagulability of a factor V Leiden carrier may increase the anchoring of the embryo to the endometrium. This higher coagulability may also increase the likelihood of thrombosis occurring in the trophoblast or early placenta, resulting in a failure of the pregnancy.

This assumption however does not completely cover the findings we made in this thesis. Paternal factor V Leiden carrier ship was found to be important, independent to the maternal factor V Leiden status. An increased fecundity (a shorter time interval between unprotected intercourse and the occurrence of pregnancy) was found if the father carried the factor V Leiden mutation, but was not found if the mother carried the mutation. It was opted that the factor V Leiden status of the fetus (inherited by the father) is significant in increasing the likelihood of embryo implantation. This remains a hypothesis, as the factor V Leiden status of the child was not retrievable. Furthermore it is unknown whether or how a blastocyst exposes

the factor V Leiden gene and if this has any effect on implantation. Maybe a factor V Leiden positive embryo has a higher likelihood to adhere or anchor to the endometrium increasing the chance of implantation; however this remains an unsubstantiated thought. Given the fact that this finding did not come from a prior defined hypothesis, it may also be a spurious association which will need further confirmation in additional studies.

This leads to a different hypothesis altogether. Possibly women suffering recurrent abortions (and perhaps even women with a decreased fecundity) have a low rather than a high threshold of accepting a pregnancy. Perhaps they have a higher likelihood of not only accepting normal embryos but also the abnormal ones that will eventually miscarry. Previously it has been stated that early (preclinical) pregnancy loss rather than failure of conception may be the principal cause for the relatively low fecundity observed in humans[45]. Per cycle the chance of fertilisation is about 70-80% however about 60% of these conceptions will miscarry, mainly before a clinical pregnancy can de diagnosed[45]. Perhaps therefore, we need to seek more in the direction of a too easy acceptance of a pregnancy. Maybe women with unexplained recurrent miscarriages have an increased tendency to accept every conception even if the early embryo has abnormalities (chromosomal or morphological) and is therefore wrongfully accepted. The pregnancy is not 'filtered' in the very early stages and can progress beyond the menstrual date when a clinical pregnancy can be diagnosed. However, as the embryo is abnormal, the pregnancy will most likely be rejected in the following weeks as the embryo will fail to develop and a miscarriage will occur. If a woman has a tendency to accept an embryo too easily, it would explain the occurrence of some recurring miscarriages and may be seen as a natural protection for having healthy offspring. One way of testing this hypothesis is to analyse all early pregnancies not only on chromosomal abnormalities but also to check for morphological abnormalities.

This hypothesis of an increased early acceptance of a pregnancy may be used not only for factor V Leiden but also for an excessive Th-2 cytokine profile. For instance diseases that are known to be Th-2 mediated with an increased IL10 production, like systemic lupus erythematosis (SLE), an increase in miscarriages is seen[46;47]. Possibly the less optimal embryo's are accepted more readily in these phonotypical Th-2 mediated diseases, subsequently ending in more miscarriages. It may well be that there is an optimal basal IL10

production that is most favourable for a successful pregnancy. A low basal IL10 production may not be enough to inhibit the Th-1 resonse well enough for the acceptance of the semi-allogenic fetus, but a high basal IL10 production may be detrimental in reducing the necessary inflammatory reaction needed for angiogenesis during the blastocyst invasion[3;40;41]. Further research using conditional IL10 knock-out mice may be an option.

6. CLINICAL IMPLICATIONS AND FUTURE RESEARCH

It is too early to translate the findings written in this thesis towards the day-to-day clinical care. It is not time yet to administer cytokines to pregnant women, as the effect will be systemic, with unknown effects on the developing fetus. Moreover, the effect of one cytokine will be diverse as it will trigger a whole cascade of other cytokines to be stimulated or inhibited, with unknown results for the pregnancy. The results found regarding factor V Leiden are also preliminary and do not alter clinical care at his stage. It may inform the clinician however, that factor V Leiden carrier ship in mother or fetus may have different consequences, and that paternal influences may be significant.

Future research should be focused on the pro-inflammatory and anti-inflammatory cytokine profile from the conception and implantation onward, with an emphasis on genetic aspects of these cytokines. Not only the maternal profile but to include the paternal profile and test the fetal profile (at birth or if possible at time of miscarriage). Concerning IL10 specifically, it would be interesting to distinguish whether different levels of IL10 mediate different effects. An interesting hypothesis may be whether a low basal rate of IL10 is necessary to increase the likelihood of implantation and whether or not the embryo is rejected is determined by differences of IL10 production at a much higher basal level.

With regard to research on miscarriages, both in relation to factor V Leiden and cytokines, it will be important to assess if the embryo/fetus was (ab)normal. An abnormal embryo may miscarry due to a number of reasons, and the balance of pro- and anti-inflammatory cytokines might be different than when a normal embryo miscarries. The assessment of the morphology of the fetus can be done by 2D or 3D ultrasound[48] or embryoscopy after a miscarriage has been diagnosed[49]. Furthermore, karyotyping of the fetal products will be important to assess if

the embryo had numerical chromosome abnormalities. Similar research could be done in fetuses that are aborted without a medical indication (abortus provoatus) and may be used as a control group.

A too 'hostile' maternal environment, but also a too 'friendly' maternal environment may induce an increase in incidence of miscarriages. These observations indicate that the processes involved in early pregnancy need to be further delineated. Subsequently it is necessary to identify the rate of limiting steps in normal physiology to understand the disturbances in fertility in the human population. Ideally this is done in animal studies first, followed by genetic association studies to find out which of these processes are rate limiting. Finally this could lead to designs of intervention trials in which patients with repeated miscarriages can be treated. Ultimately this could lead to less suffering of couples experiencing early pregnancy problems.

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