

Intra-amniotic antigen installation for safe fetal growth disruption

Introduction: Every year 21.6 million women die due to unsafe abortions most of which are conducted unethically. Out of this 21.6 million women, 18.5 million women die alone in the developing countries and a further 47,000 women from complications of unsafe abortions each year. According to WHO, close to 13% of unsafe abortions is the main cause of all maternal deaths. (http://www.who.int/reproductivehealth/topics/unsafe_abortion/magnitude/en/)

The two most common methods for inducing abortion are instrumental/surgical evacuation through the vagina and medical induction/stimulation via uterine contractions. Dilation and curettage (D & C) is normally performed on less than 14 weeks fetuses, Dilation and evacuation on 14-24 weeks fetuses and for less than 9 weeks fetuses MVA or manual Vacuum aspiration can be conducted. If the fetus is more than 9 weeks then electrical vacuum aspiration or EVA technique is used involving the attachment of a cannula to an electrical vacuum source. (<http://www.msdmanuals.com/professional/gynecology-and-obstetrics/family-planning/induced-abortion>)

Other uterine surgeries include hysterotomy ligation or even hysterotomy, however, they are considered as a last resort. The shortcomings of the surgical abortion intervention are the loss of blood due to excessive bleeding, infection requires another small scale surgery leading to morbidity and mortality although in very few cases in developed nations but alarmingly high in developing countries. (<http://www.msdmanuals.com/professional/gynecology-and-obstetrics/family-planning/induced-abortion>)

Medical induction of abortion can be conducted on fetuses which are <9 weeks or >15 weeks old. 25% of all abortions in the US alone are conducted by means of medical abortions which include both the instillation of progesterone blocker mifepristone and Prostaglandin E1 analog and misoprostol. However, there are many side effects that are normally associated with using prostaglandins and progesterone blockers in a medicine induced abortions like nausea, vomiting, diarrhea, hyperthermia, bronchospasm and decreased seizure threshold jeopardizing the mother's health in some cases. (<http://www.msdmanuals.com/professional/gynecology-and-obstetrics/family-planning/induced-abortion>)

The above-mentioned processes require a lot of trained physicians, well-maintained infrastructures, should be conducted after ethical and legal permission from the mother and strictly following the laws of the land. More importantly, the good being and good health of the mother with subsequent follow-up is an utmost necessity. The prevalence of quack doctors in the periphery regions of developing countries that are not well trained and doesn't possess the necessary qualifications to undertake such complicated procedures often put the mother's health at risk and jeopardy. Unfortunately in many poor countries where due to social constraints and taboos pregnancy termination is at large and conducted illegally and unethically, many mothers and families are exploited in exchange for monetary favors. Also, cost is a very important factor in undergoing medically induced abortions as many cannot even afford the cost of Prostaglandin or Progesterone blockers. Also, surgery as already discussed brings in a lot of uncertainties in the mother's health and safety in the longer run.

Therefore keeping in mind the above limiting factors prevalent at large in poor countries, Niranjana Bhattacharya and his group led the discovery and search for a safe, cheap, effective, mid-trimester

abortifacient between 1978 to 2002 like tetanus toxoid and BCG. BCG couldn't be continued due to unavailability of ethical permission but encouraging results were observed by the above group in this new field of modern medicine using intra-amniotic instillation of tetanus toxoid in inducing abortions in mothers who wanted to undergo abortion due to social and other reasons after giving their ethical and legal consent to the research team including no objection from the Institutional Ethics Committee

Further, this group of medical researchers from Calcutta headed by Niranjana Bhattacharya also revealed many hitherto unknown secrets about the growth and development of the human fetus up to 20 weeks. (1) That the fetus, even at a very early stage of growth can react to an antigen, even in the sterile environment leading to embryopathy changes up to 9 weeks or the pre-immune phase 10-16 weeks in a non-specific manner resembling an acute non-specific infection or the hypo-immune phase from 17 weeks onwards is totally a new finding. These findings may have serious implications for future medical research. (1)

Global mortality from tetanus toxoid is 1 million of which 880,000 deaths are the recorder for neonatal tetanus. (2) Adequate popularization of this simple, safe, cheap and easily procurable, effective method of abortion using BCG and tetanus toxoid can combat the criminal abortion menace and the cost as it is extremely cheap and can be even afforded by the poorest of the poor in developing nations, if confidentiality is maintained (92.8% will abort in one month, as shown by various experiments by the research group in Calcutta). In the developing countries, in particular, local health departments can take the initiative by organizing camps and workshops and encouraging the participation of social workers, nurses, midwives, village level quack doctors or barefoot doctors, pharmacists or even criminal abortionists agreeable to accept change, from any sphere of life in urban, semi-urban and rural areas. (4)

Fetal Immune system:

It is well known that the function of the immune system is to protect the individual from an invasion of foreign antigens by distinguishing the self from the nonself. A normal immune response relies on the careful coordination of a complex network of specialized cells, organs and biological factors necessary for the recognition and subsequent elimination of foreign antigens. (5) An exaggerated immune response can result in the hypersensitivity to foreign antigens with resultant tissue injury and the expression of a variety of clinical syndromes like Type I (IgE mediated) immediate hypersensitivity reaction, Type II (IgG, IgM, complement activation) antibody mediated cytotoxicity reaction, Type III (antigen-antibody complex and complement activation) immune complex reaction and Type IV (lymphocyte) delayed hypersensitivity reaction. Two other mechanisms are also proposed Type V and Type VI of which Types I to III are humoral immune responses and Type IV response results from initial sensitization and subsequent antigenic challenge. (5) This kind of antigen elimination through the cellular or humoral process is integrally linked to the inflammatory response in which the cellular messengers (cytokines) and antibodies trigger vaso active inflammatory mediators. (5) This inflammation has got a positive role i.e. to promote efficient elimination of foreign antigens and to prevent uncontrolled lymphocytic activation and antibody production and also negative and deleterious effects. In the case of the growing innocent embryo or the hypo immune human fetus, all the factors responsible for inflammatory response and its coordination are also developing at that stage of gestational maturity. Hence, poor coordination may lead to the negative effect of inflammation due to inappropriate activation of a growing fetal system thus causing dysregulation and perpetuation of the inflammatory process which leads to tissue damage and organ dysfunction and the death of the fetus with massive hemorrhage and congestion of all the viscera up to 16 weeks. (5) From 17 weeks onwards, due to the progression of the ontogeny of the human immune system, the reactions are less dramatic, typified by the characteristic changes

of mononuclear invasion, less hemorrhage and congestion and damage to the architecture of the growing human fetus. (5)

Reaction of the pre-immune or hypo-immune fetus (up to 20 weeks):

Earlier investigations reported the implications of congenital infections in case of maternal syphilis (2, 6) and rubella in the human system, rubella infection and blue tongue virus infections in lambs and lymphocytic choriomeningitis virus in mice. (7, 8,9,10, 11, 12) They suggested that the developing fetus of many mammalian species is able to mount a highly efficient immunological response to the agents responsible for congenital infections.

The present research is the first documented work (Medline search verification, 24 October 2004) on the intra-amniotic direct antigenic challenge to the human fetus showing the reactions of the developing pre-immune and hypo-immune fetus; however there are certain obvious differences with the congenital infection scenario. (1)

In the case of congenital infections, the placental barrier is formidable and unless this is broken, access to the fetal compartment or fetal organs is not possible. (13) The sequence of events: maternal severe infection, the involvement of placenta and loss of placental function-the fetus is involved. The damage to the fetal system is due to the resultant action of toxins/chemicals liberated by the living or dead/offending organisms and their interaction with the growing fetal system as well as the primitive non-specific to specific developing immunological defense against the offending organisms.

In a case of this medical research by Niranjan Bhattacharya et al., with different antigenic challenges the placenta is never damaged primarily. It is only damaged secondarily to fetal damage or death. Hence the changes are primarily the result of fetal changes due to the interaction of the fetal system with the antigenic stress. On the basis of macroscopic and microscopic studies, similar hitherto unknown fetal reactions to an antigenic invasion of the fetal system were observed. However, the reactions with glutamate BCG (up to 15 weeks) were found to be more. (14) Here fetal death and fetal dissolution were observed of varying degrees without abortion, a hitherto unknown phenomenon. The group on further analysis revealed the sensitivity of the growing human embryo to a fetus in the light of different experiments with the intra-amniotic challenge. (1)

Intra-amniotic challenge with tetanus toxoid:

Intra-amniotic tetanus toxoid antigen challenge in the teratogenic phase (up to 9 weeks) to the pre-immune phase of human fetal growth (10-16 weeks) leads to massive haemorrhage. Also, congestion of all the viscera and changes in the growing architecture of the fetal organs, eventually leading to death and abortion of the fetus. (15) Tetanus toxoid challenge at the hypo-immune phase (17 weeks onwards) of human fetal growth leads to mono cellular cell invasion, haemorrhage, thrombosis in all the organs including the placenta where there are additional features of villitis and deep subchorionic fibrosis, eventually leading to abortion in a less dramatic way with mostly living fetuses. (16) Therefore the above results concluded that even in the sterile environment of the amniotic fluid, the developing fetal system can also react to any antigenic challenge; however the reactions are dependent on type/route/virulence/dosage of the antigenic assault. It is believed that this at least will partially explain the apparent benign presence of treponema palladium over the very early fetus (6). On the basis of research conducted over the last 3 to 4 decades it is felt that a little more presence of treponema antigenic load in a very young fetus will abruptly change the entire scenario from benign presence to a cruel presence triggering death cum deformity and abortion of the fetus depending upon the stage of fetal growth from the teratogen to the pre-immune phase or hyperimmune phase of the fetus due to the crossing of the critical mark of fetal susceptibility cum tolerance of the antigen load. (17) This may result in an exaggerated and hypersensitive type of acute response in the early weeks (up to 16 weeks) and a chronic response in

the later weeks (above 17 weeks) as seen with different antigenic challenges which is evident clinically in the case of the aborted fetus, depending on the gestational age of the fetus. (1)

Clostridium Tetani:

Clostridium tetani are the causative agent of tetanus and is one of the important species of the genus clostridium, comprising of spore forming gram positive anaerobic bacilli. Two products liberated by C. tetani are the classical neurotoxin (tetanospasmin) and hemolysin (tetanolysin). The hemolysin is heat labile and inactivated by oxygen. All the symptoms in tetanus are attributable to an extremely toxic neurotoxin. In a case of an adult/baby, the number of Lf of toxoid should not exceed 25Lf for primary immunization as recommended by the World Health Organization (WHO). (1) There is no specific time period of pregnancy when women should be immunized against tetanus. In those countries where the risk of tetanus neonatorum is low, usually the immunization is deferred during pregnancy. It has been seen that immunization during the fifth and eighth months of pregnancy results in the formation of antibodies in the infant and also enhances the response of these infants to subsequent immunization. (1) This phenomenon has been termed as transplacental immunization. (1) Though extremely rare, certain adverse effects of tetanus vaccination have been reported like swelling, redness and pain up to 10 days at the site of injection. (1) Other systemic reactions like pyrexia, myalgia, malaise, acute anaphylaxis, peripheral neuropathy, elevated IGE level and elevated Anti-A and Anti-B antibodies are also implicated. Extremely rare reactions have been observed in individuals who had high levels of circulatory anti toxin and in whom bolstering was attempted. Tetanus toxoid has been reported to increase Anti-A and Anti-B antibodies owing to the traces of blood group antigen in the vaccine. Immunization of patients (pregnant) with the aim of preventing neonatal infection may, therefore, increase the risk of hemolytic disease of the newborn. (2) With this backdrop of information in mind, the researchers used tetanus toxoid as an intra-amniotic injection to study its interaction with the fetal growth and development in utero. The research group from Calcutta performed this novel intra-amniotic disruption of Human Fetal growth in 688 cases of abortions which were all conducted after the mother consent, ethical and legal clearance and following the laws of the land. (18) The abortions were mediated by a single intra-amniotic injection of 2 cc tetanus toxoid. The overall success of injecting this material in the fetus resulted in 92.80% of the abortion of the fetus. After the cutoff period of 7 days, the following post intra-amniotic 2 cc injection the abortion rate fell down to 52.5% and 72.4% after 14 days cut off period. On the 21st day, the cut-off rate became 86.2% and 92.8% at 30 days. Therefore, the researchers observed that with a single 2cc intra-amniotic membrane injection of tetanus toxoid there was 94.74% abortion in 8 to 11 weeks group and 94.05% in the 12-15 weeks if the cutoff period was for a month. It was further revealed from the research, that the induction-abortion interval varied widely with a progression of gestation to 16-19 weeks or more and it increased gradually from the mean induction-abortion interval. The success rate of a single dose of intra-amniotic injection of 2 cc schedule progressively comes down to 88.61% from 94.74 % in the 8-11 weeks group and 73.67% eventually in the 20 weeks group. (1, 19)

The group further studied the effects of intra-amniotic tetanus toxoid injection varying from ½ to 4 cc all single doses. (1) It was revealed that the abortion rate on the 7th day of ½ cc of tetanus toxoid is only 60% compared to 1 cc on the 7th day at 71.42 % and 37.5% on the 7th day with 1.5 cc. 2 cc tetanus toxoid resulted in 47.2 % on the 7th day followed by 60% on the 7th day with a dose of 3 cc. (1,20, 21) 4 cc yielded 66.6% failure rate on the 7th day. On analysis of the results on 14th day it was revealed that with ½ cc of single dose of intra-amniotic tetanus toxoid, only 30 % underwent abortion, with 1 cc the failure rate on the 14th day was 42.85%, with 1.5 cc it became 25%, 2 cc yielded 27.6%, 3cc resulted in 30% and 4 cc on the 14th day resulted in 33.3% of failure on the 14th day. Multiple injection dose regimes were also followed and it was observed that the cumulative success rate is higher in multiple doses on the 14th day as against single dose on the same day. (1, 20, 21) Further discussions with Prof. Arnold Klopper of the Royal College of Obstetrics and Gynaecologists and visit Calcutta on 18th January 1980, the group was suggested that in order to reduce the

unpredictability of fetal stimulation intra-fetal injection can be an alternative method although this procedure also involved certain technical limitations like fetal movement, some regurgitation of the antigen to the amniotic cavity depending upon the site of injection, needle bore and fetal movements and inadvertent injury to fetal organs like fetal liver, lungs while giving intra-fetal injection to the fetal back muscle.(1,22, 23, 24, 25 ,26)

Analysis by this same research group revealed using intra-fetal injections, 50% abortion on the 7th day, followed by 83.335 abortions on the 14th day which was the highest abortion rate and better even with multiple intra-amniotic injections where the rate of abortion was 79.1 % on the 14th day. The addition of fetotoxic or immunomodulatory substance like Vitamin A in the dosage of 300,000 IU injected with tetanus toxoid revealed 70% cases of abortion on the 7th day and a cumulative 40% abortion on the 14th day. With oral levamisole, 150 mg 58.33% of the fetus went abortion on the 7th day and 25% cases failed on the 14th day. This was conducted to observe the role of the T cell stimulation or modulation of T cell function. Also, intra-amniotic injection t fetuses with tetanus toxoid before 20 weeks of development showed 62.5% of cases aborted within 7 days as against 75% within 14 days. (1, 27, 28, 29, 30, 31, 32, 33, 35, 36)

Intra-amniotic challenge of the fetus with BCG:

A Recent meta-analysis concluded that the BCG vaccination is highly effective in reducing the risk of tuberculosis for several years for newborn or infant immunization by at least 50%.(37) Although BCG vaccinations have been safely used in new borns throughout the world, the safety of BCG vaccination in immune-compromised patients, such as HIV-infected patients, has not been verified and the patients may be at a risk of developing disseminated disease. (38)

The research Group further experimented with the use of BCG. They have shown the effect of injection of single BCG (1cc) intra-amniotic ally on the developing fetal system with the gestation of 11-15 weeks of pregnancy. To their utter surprise, there was no abortion. However there was a progressive diminution of the size of the uterus and on the 14th day of hysterotomy, the uterus was found to be practically free of the uterus. Same experiments were repeated again but the results remained the same on the 14th day of BCG instillation. 26 such further cases were conducted with gestation varying between 11-15 weeks and further studies on more than 15 weeks could not be conducted due to ethical committee restriction imposed on the use of BCG experimentation on the plea that persistence of placenta or any other fetal tissue in the maternal system may have the possibility of inducing hydatidiform mole or choriocarcinoma change to the mothers in the long run. (37)

However, here the principal question arises that why there was no abortion in the group and instead there were auto-absorption and dissolution of the fetus. The possibility that the fetal unusual immune response, due to stimulation of the developing fetal cellular immunity was causing this death cum dissolution could neither be supported nor rejected because of the dearth of information in this field, and because ethical restrictions would not allow the research group to proceed further on sequential amniotic fluid/fetal cytokine study.

Challenge of the fetus with other agents:

The research group further tried other antigens like double antigens, i.e., 5 cc diphtheria toxoid and 0.5 cc pertussis in intra-amniotic single 1 cc single shot injection schedule. Here 57.15% of success was observed 57.15 % on the 7th day of stimulation and 71.43% on the 14th day of stimulation. Generally, the combination potentiates the action. However, the result is not grossly different from our tetanus toxoid study. (1)

Using a non-specific antigen-like 20%, of 2cc BVS was utilized in 14 cases with the gestation of 11 to 18 weeks (mean gestation 14.6 +/- 1.2 weeks). Here it was noted that 42.86 % abortion occurred within seven days and cumulative 71.43 % abortion within 14 days. (1) Hence, the group concluded that the growing human fetus can act or rather react with antigen in different ways, depending on dosage and the type of antigen. However the action is not specific to bacterial antigens only and the fundamental question which remains to be cleared is the behavior of antigenic challenge on a growing human fetus can act or rather react with antigen in different ways, depending on dosage and the type of antigen. These methods of abortion can be also due to the behavior of antigenic challenge on a growing human fetus which is actually passing through a pre-immune or hypo-immune state, due to lack of proper immune system development. At this stage of development inside the protective sterile environment provided by the maternal uterus, the fetal thymus, and its supporting systems are engaged in gradually equipping its primitive guards (immune system) for the home ministry work, i.e., differentiating between its own people and the enemy, i.e., own antigen and foreign antigen. (1)

On further injection of 10 ccs of maternal blood intra-amniotic ally with a mean gestation being 13.4 +/- 1.2 weeks, abortion was noted in 40 % of the cases within 7 days and 70 % of the cases within 14 days. Buffy coated WBC was injected from 10 cc maternal whole blood prepared freshly and injected intra-amniotically. Here, too, 31.25 % cases aborted within seven days and 50 % of the cases aborted within 14 days. (1)

The basic question is to what extent can fetomaternal transfusion trigger abortion in a growing fetus? From the experience of chorionic villus sampling (CVS), certain investigators have suggested a basic prevalence of maternal-fetal transfusion (MFT) at the first trimester of pregnancy. Various theories have been developed on the pathogenesis of vascular disruptive syndrome following CVS. Most of them consider etiology of vascular incidence by thrombosis of the sampling site with subsequent embolization of the fetal vessel, trophoblast embolism, hypoperfusion of the fetal circulation following voluminous fetomaternal circulation or release of vaso active substances. (38, 39, 40, 41) Other concerns are damage of the extra-amniotic tissue, especially the fetal membranes and subsequent limb malformations due to amniotic bands or oligo-hydramnios (42). The possibility of entrapment of the fetal limb to the extra coelomic space has also been suggested (43). In animal experiments, clamping of the uterine vessels, handling of the uterus or maternal cocaine administration resulted in hemorrhage and necrosis of fetal limbs (44, 45, 46). Also experimentation with maternal whole blood injection in the amniotic cavity, it could not be gathered how much out of the 10 cc of maternal blood actually cause a load to the fetal circulation because it could not be assessed properly. But in a case of a growing fetus at around 12-13 weeks pregnancy the total blood volume is only 2.7 cc to 4 cc (25), which increases progressively during gestational maturity. This can be considered without any vascular communication for further compensatory fetomaternal hemorrhage due to sudden vascular load. However, this can also have an immune damage potentiality for the fetus. A possible mechanism could be agglutination of the fetal erythrocytes or erythroblasts in early pregnancy. (27) A more probable mechanism might be a graft versus host reaction against fetal endothelial surface antigens. (28), as some experiments by the group suggests that apart from RBC, other maternal WBC antigen can also cause havoc in a growing fetal system if there is a sudden WBC perfusion from the mother to the growing fetus. There was abortion if buffy coated leucocytes separated from freshly drawn 10 ccs of mother's blood were injected through the intra-amniotic route in 31.25 % cases as evident on the 7th day and 90 % cases within 14 days. (1) Further, 5 cc of amniotic fluid of other allogenic mothers, was drawn freshly and injected once intra-amniotically, in gestationally randomized cases (both for drawing amniotic fluid as well as for

injecting amniotic fluid) and 33.3 % of cases aborted within 7 days and 58.34 % cases aborted within 14 days. (1)

The amniotic fluid is alpha fetal protein (AFP) and this protein is embryo-specific or developmental substance-feto specific proteins, transient proteins, which are synthesized only during a particular period of growth. These disappear or persist only in trace amounts during adult life unless their synthesis is re-stimulated by some unusual conditions such as malignancy. (47, 48) This alpha fetal protein (AFP) accounts for less than 0.5 % of the total protein present in the amniotic fluid, yet nearly all the alpha fetal protein is of fetal origin. (49, 50) In a series of experiments it has been shown that AFP can inhibit the primary and secondary cellular response in splenic plaque forming cells to sheep RBC; also, in some cases, it can suppress thymus-derived T cell dependent functions of allogenic and mitogens induced lymphocytic transformation. Elevated levels of alpha fetal protein can depress the maternal immune response and delay the rejection phenomenon of the fetus. It also reduces the capacity of the fetus and the newborn infant to respond to antigenic stimulus. With the disappearance of AFP at birth, the immune suppression state is lifted. (50) TA Waldmann and KR McIntire reported elevated levels of AFP state above 30 ng/ml (44-2800 ng/ml) in patients with ataxia telangiectasia (immune deficiency state with absence of gamma globulin, recurrent infection, telangiectasia of conjunctiva and skin, cerebellar ataxia, apraxia of ocular movements, etc.) (51) H Cohen et al have shown the absence of maternal antibody to fetal alpha fetal protein. (52) Why a mother does not produce antibody to fetal alpha fetal protein is not known. (53) Hence, contrary to its role as an immune-suppressive or immunomodulatory, it can participate in triggering the abortion process either directly or indirectly by other elements of the amniotic fluid, i.e., suspended fetal cells or gamma fetal protein, etc., in relatively trace amounts, if injected into another allogenic mother's amniotic cavity.

Maturity of the fetal immune system:

The proliferation of fetal T cells to different mitogens can be seen as early as 10-12 weeks. However, mixed lymphocytic culture (MLC) response by helper fetal T cells starts as early as 14 weeks gestation of the fetus and the MLC response of the T helper cells match the adult response from 18 weeks gestation of the fetus. The function of CD8 T cytotoxic cells which recognize non-self antigens presented by MHC class I molecules on the surface of target cells depend on the production of interleukin-2 by (TH1) helper T cells. (54) This target cell killing by in vitro cell-mediated lympholysis assay is not seen until 15-22 weeks gestation and the function is far less compared to the adult response of CD8 T cells until birth. The function of natural killer cells, though traced from six weeks gestation is present normal numbers in the second half of gestation, but with less or diminished cytotoxic activity even in the neonatal period. Though some complement component can be seen from 6 to 14 weeks in the human fetus, even in newborns the activity of the alternate pathway is moderately reduced. The B cell function of a growing human fetus is CD 40 dependent through lymphokines modified pathways. These B cell functions are poorly developed during the fetal and the newborn period as memory B cells and plasma cells are rarely detected in non-infected neonates. (54)

Normally in a mature immune system, there is an immune-neuroendocrine network with regulatory interdependent circuits where feedback by cytokines augmenting the production of corticosterone is important because it shuts down TH1 and macrophage activity and the positive role of estrogen for a more active immune response in females relative to males. But in a growing human fetal system if there is an antigenic challenge, for examples with tetanus toxoid or BCG, the mystery pertaining to how does the immature immune-neuro-endocrine coordination modulate itself such a challenge in a so-called hyperimmune state of the growing human fetus still remains at large. (1)

In this connection, it is worth mentioning that in the case of multiple injections of tetanus toxoid through the intra-amniotic route, there is a marginal improvement in the cumulative abortion rate, but if the memory cell population was higher, the effect would have been more dramatic. The proliferation of fetal T cells as a result of stimulation by various mitogens can be seen in as early as 10-12 weeks gestations and in mixed lymphocytic culture (MLC) by 14 weeks. (54) However, responses do not reach that of adult T cells until approximately 18 weeks. Activation of neonatal CD4 T cells synthesizes normal amounts of interleukin -2 but markedly depressed amounts of Interleukin-3,4,5, gamma interferon, and granulocyte-monocyte colony stimulating factor (GM-CSF) when compared to adult T cells. This apparent Immune deficiency of neonatal T cells reflects more of immunological immaturity rather than an intrinsic defect. The ability of cytotoxic T cells (Tc) to kill target cells in vitro cell-mediated lympholysis is not seen until 15 to 22 weeks and even then it is not comparable to that of the adult CD8 + T cells until birth. (54) Functions of the NK cells, a lymphocyte subset can be identified with the expression of CD 16 and/or CD56 and absence of CD3. These NK cells develop normally in the absence of the thymus and most are CD2+ and CD7+ and some are even CD8+. However in contrast to T cells which recognize foreign antigens in association with self-MHC molecules, the NK cells can recognize the pathogens in the absence of self MHC expression. (54) The cytotoxic activity of NK cells is initiated by the engagement of antigen and IgG with CD16 which is known as antibody-dependent cell-mediated cytotoxicity or it is initiated through contact with target cells lacking the expression of self MHC antigens. (54)

Cells with NK phenotype have been detected as early as 6 weeks gestation and are present in normal numbers throughout the second half of gestation; however neonatal NK cells have definitely diminished cytotoxic activity. In a mature immune system, the complement system operates through (a) classic and (b) alternative pathways through interaction in a cascade fashion which plays an important role in cell lysis opsonization and chemotaxis. The (a) classical pathway operates through the antigen-antibody complex and the phylogenetically older (b) alternative pathway is not dependent on antigen-antibody complexes but occurs with the exposure of polysaccharides, endotoxin, and other structures. These complement components are produced principally by the hepatocytes and to a lesser extent by macrophages. The complement components do not cross the placenta. In growing human fetal systems some complement components are seen between 6 weeks and 14 weeks. (55, 56) In newborn infants, the classical pathway components and activity are comparable with the adult level but the components of the alternative pathway are markedly reduced and are less than 20 percent of those of a normal adult. This state of relative complement deficiency continues throughout the entire gestational period and extends to the neonatal period of birth. (55, 56)

Changes in the fetal organ architecture post-fetal challenge with antigens:

In the case of the aborted fetus (14 weeks), 72 hours after a 2cc intra-amniotic tetanus toxoid challenge the researchers observed total disruption of liver architecture with extensive hemorrhage, congestion, and edema in the background with cellular infiltration. (1) In the case of the 18 weeks fetus, the disruption is relatively less with mononuclear infiltration and less edema. In the case of the nonaborted fetuses with a blank antigen like adsorbent/saline control 14 weeks, there is no disruption of architecture or cellular infiltration or edema, nor is there any hemorrhage as is evident from the histological study. (1)

In the case of the growing spleen challenged with blank antigen, i.e., normal saline, etc., histologically there is no change. The antigen challenged fetus (tetanus toxoid), though not aborted spleen specimen showed disruption of architecture at places with endothelial and macrophage cells

seen in the background of tissue edema and loss of follicles. This disruption is less prominent in the 28 weeks anencephalic fetus.(1) This disruption is highest In the case of abortion.(1) In case of kidney and supra renal, antigenic challenge (tetanus toxoid) induced abortion 14 weeks and 18 weeks against non-abortions 14 weeks and 28 weeks showed disruption of architecture in the background of cellular infiltration, hemorrhage, congestion, edema in 14 weeks which was less prominent in the 18 weeks abortus with mononuclear cell infiltration and thrombosis. (1) In case of skin, similar architectural disruption was noted in the aborted fetus 14 weeks with excessive edema, cellular infiltration after antigenic challenge with tetanus toxoid with respect to non-challenged or controlled fetuses.(1)

In the placenta, the changes were similar varying degrees of architectural disruption with edema, congestion, hemorrhage and cellular infiltration of the organs, which was less prominent in the non-aborted fetus. But the observation that antigen challenge causes some histological changes becomes quite obvious when we compare the antigen-challenged architecture with the antigen blank normal saline challenged architecture of the growing human fetus. (1) A study of the placenta is very important because it is the only tissue where maternal and fetal responses can be studied simultaneously. A comparison of the aborted placentas at 18 weeks and non-aborted placentas after antigen challenge and blank antigen (normal saline + preservative) challenge showed certain definite changes. (1)

Extensive infiltration of mononuclear cells thrombosis edema, interstitial hemorrhage and villites were seen in the 18 weeks aborted fetal placenta. Hemorrhage congestion and cellular infiltration were less prominent in the antigen-challenged non-aborted fetus at 14 weeks and 28 weeks (anencephaly). Villites was consistently present with focal areas of chronic inflammation with mononuclear cells, and areas of fibrinoid necrosis in the chorionic villi in all cases of antigenic stimulation through the intraamniotic route. Various investigators have suspected that villites represent the histopathological evidence of fetal graft rejection; however, immune, histochemical and other studies have not differentiated between graft rejection and a normal immune response against an infection agent. (57) However, the trend of research by the same investigator suggests that (a) villites are initiated by the fetus : one never encounters it in the first trimester when the fetus lacks immuno competence; (b) lesions indistinguishable from villitis of unknown etiology have been reported with diverse placental infections including cytomegalovirus, enterovirus, mycoplasma and syphilis; (c) the morphological response of a fetal response to a foreign antigen is best seen in early villites where inflammatory cells appear to be migrating from the fetal capillaries to the villous trophoblast surface : this indicates that although invasive maternal cells may occupy an advanced lesions, the early or primary inflammatory response is fetal. (58)

Vaccination of the Unborn:

Using the above concept, the group led by Niranjan Bhattacharya also started a new concept in vaccination of the unborn. In developing countries, unborn infections are an important cause for a high rate of mortality and morbidity in young infants. Current immunization strategies focus on preventing infections by vaccinating the infant at around 2-4 months, however, this strategy fails to prevent important infections of the newborn in the first month. (59) Hepatitis B vaccine can safely prevent neonatal infection and long-term sequelae including cases of pregnant women who are vaccinated with tetanus toxoid to reduce tetanus infection in neonates. (60, 61)

Advantages of vaccination in a term baby it that it has antibody at birth and there is active immunity in the mother. However, disadvantages include decreased passive antibody in premature infants, passive immunity in the newborn baby which wanes progressively within 6 months.

The group Bhattacharya et al. has the first global experience in intra-fetal vaccination attempts before 20 weeks of gestation. The group through their experience has suggested that the vaccination of the unborn via the intra-amniotic or intra-fetal route is not feasible as it has serious adverse effects as observed in the histology of 14 and 28 weeks non-aborted fetuses. Also, there is a poor antibody response and there is the abortion but no fetal death reported in this series of growing fetus above 18 weeks. This toxic non-specific reaction can be due to the reduction in the antibody formation and down-regulation of another immune coordination system as the fetus is still immature. Therefore the group of scientists concluded that any immune-vaccine intervention before 20 weeks might prove to be toxic to the survival of the fetus.

Other changes post vaccination showed an increase in essential fetal liver enzymes including the weight of the fetal thymus, spleen, cell number and liver when compared with the blanked/control fetus. These symptoms were all hallmark of Graft versus Host Syndrome. (62) However, the research group did not believe in such GvHD reactions in fetuses less than 16 weeks old as it is still in an immature or pre-immune stage prior and antigenic stimulation normally happens post 17 weeks. The researchers further believe that this abortion pattern can be due to the auto immune reaction or even unnamed inflammations the basics of which are still to be elucidated.

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See also:

Abortion

Immune tolerance in pregnancy

Human Fetal Tissue research in Regenerative Medicine

External links:

Immune system development:

https://embryology.med.unsw.edu.au/embryology/index.php/Immune_System_Development

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