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Review Article

Immunobiology and immunotherapy of gestational trophoblastic disease

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ABSTRACT

Gestational trophoblastic diseases are a heterogeneous group of pregnancy related tumors that show extensive metastatic spread but are readily responsive to chemotherapy. This one of a kind treatability of gestational trophoblastic tumors may to some extent be inferable from a host immunologic reaction to the paternal antigens that are expressed on the trophoblastic cells. In this review, we evaluate the current cognizance of immunobiology of gestational trophoblastic diseases and also establish the immunologic behaviour of gestational trophoblastic diseases which should be researched further in order to gain a better understanding of the aetiology of these neoplasias. This will further help structuring immunotherapeutic methodologies for their treatment.

1. Introduction

Gestational trophoblastic disease (GTD) is a heterogenous but interrelated group of gynecological tumors that are defined by aberrant trophoblastic proliferation, ranging from premalignant conditions such as complete and partial molar pregnancies to malignant conditions like invasive mole, choriocarcinoma, placental site trophoblastic tumour (PSTT) and epithelioid trophoblastic tumor.[1,2]

There are wide variations in the occurrence of GTD all throughout the world. It accounts for less than 1% of all gynecological malignancies.[3] In most parts of the world, hydatidiform moles occur in about 1 in 1000 pregnancies, with complete moles occurring in 1–3 per 1000 pregnancies and partial moles appearing in 3 per 1000 pregnancies.[4] Approximately 18,000 women per year, are diagnosed with gestational trophoblastic disease and chemotherapy and surgery form the mainstay of their treatment. Chemotherapy resistance and rare types of GTD like placental site trophoblastic tumour are the main cause of treatment failure and poor outcome in at least 5% of these patients who succumb to this disease.[5]

An empty ovum is fertilized by a haploid sperm or two sperm spontaneously, resulting in a complete hydatidiform mole. It is diploid with 46,XX or 46,XY karyotype. When a normal ovum is fertilized by two sperm, a partial H mole develops with 69,XXY triploid karyotype.[6] The two confirmed risk factors for gestational trophoblastic neoplasia are extremes of maternal age and previous molar pregnancy (GTN). Women aged <20 years and >35 years have 1.9 times higher risk of developing complete mole than women of age group 21–35 years. The risk further increases to 7.5 times for women more than 40 years of age.[7] Prior miscarriage, animal fat food consumption, ovulation induction for fertility, women with blood type A or AB, long-term use of birth control pills, and family history are among the additional risk factors.[8]

Treatment of GTN is decided as per stage of the disease and WHO risk factor scoring system(Table 1).[9]

Single-agent chemotherapy with methotrexate or actinomycin D can be used to treat patients with Stage I, II, or III who have a risk score of 7. Patients with stage IV cancer and stage II-III cancer with a score of 6 or above require multiagent chemotherapy such as the EMA-CO and EMA-EP regimens (E-etoposide,M-methotrexate,A-actinomycin D,C-cyclophosphamide, O-vincristine and P-cisplatin).[10] Patients with prognostic values of 5 or 6 are more likely to develop single-agent drug resistance, necessitating multiagent chemotherapy.[11] Despite the fact that GTN is chemosensitive, 25% of high-risk GTN patients develop resistance to first-line chemotherapy or relapse following treatment, mandating salvage chemotherapy, which is usually exceedingly toxic.[12] As a result, novel therapeutic options for chemoresistant GTN are urgently needed. Immunotherapy has the potential to be a game

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Table 1
WHO Risk scoring system.[9].

Prognostic factors	Score 1	Score 2	Score 3	Score 3
Age (year)	<40	≥40		
Antecedent Pregnancy	Mole	Abortion	Term	
Interval months from end of index pregnancy to treatment	<4	4-<7	7-<13	≥13
Pretreatment serum HCG (IU/L)	$<10^3$	10^3- $<10^4$	10^4 - $<10^5$	$>10^5$
Largest tumor size, including uterus (cm)	<3	3-<5	≥5	–
Site of metastasis	Lung	Spleen, Kidney,	Gastrointestinal	Liver, Brain
Number of metastasis	–	1–4	5–8	>8
Previous failed chemotherapy			Single drug	2 or more drugs

changer in this situation.

2. Immunobiology of gestational trophoblastic disease

The molecular profile of GTD has been widely investigated over the last decade, paving the way for immunotherapy as an emerging treatment strategy in the management of GTD. The immune response of the host to paternal antigens produced on trophoblastic cells appears to be crucial in the treatment of GTD.[13] The sensitivity of GTD to chemotherapy is also partly attributable to this mechanism.

The main characteristics that distinguish GTD from other solid malignancies are that it originates from the placenta and is associated with gestation, i.e. pregnancy, and that, like normal trophoblastic cells, it contains material originating from the paternal genome, which acts as semi-allogeneic implants. It has the potential to elicit a strong antigenic response from the maternal immune system. The GTDs outweigh the maternal immune system and outpace placental and peripheral immunity, allowing them to evade immune surveillance by unrestricted proliferation, invasion, and diffuse metastases.[14] Taking into consideration the immunological oddity of GTD, many novel treatment options are emerging including immunotherapeutic strategies.[15]

The placenta villi are made up of three sheets of segments, each with a different cell type: (1) Trophoblast cells that cover the entire villous tree's surface. They are further subdivided into cytotrophoblasts, which make up the trophoblast's innermost layer and are responsible for syncytiotrophoblast cell growth. The outermost layer of the villous tree is formed by syncytiotrophoblast is a layer of epithelial cells that forms the outermost layer of the villous tree and which is in direct contact with maternal blood within the intervilland is in direct contact with maternal blood within the intervillous space; (2) Mesenchymal cells, mesenchymal derived macrophages (Hofbauer cells), and fibroblasts that are located within villous core stroma between trophoblasts and foetal vessels. Hofbauer cells synthesise Vascular endothelial growth factor (VEGF) and other proangiogenic substances in the placenta that start the process of vasculogenesis[16]

Fetal antigens are present on villous trophoblasts and when they come in contact with the maternal circulation, it results into their exposure to maternal immune cells.[17] Further the extravillous trophoblast (EVT) cells, also called interstitial trophoblasts are a type of cytotrophoblast cells that are present at the tip of placental villi.[18] They also proliferate, metastasize and invade the placental decidua layer and maternal peripheral circulation. The trophoblastic migration, which can resemble tumour invasion and is tightly controlled inside the myometrium, is regulated by both stimulatory and inhibitory signals. The development of GTD is linked to an imbalance of stimulatory and inhibitory factors.[19,20]

Table 2 enumerates the various antigens presented on the surface of the villi which show how immune mechanism come into play in GTDs and these antigens can act as a potential target for the development of treatment modalities that can be used in the treatment of GTDs in the future.[21–38]

GTD also has a mechanism to escape the humoral immunity of the host by following immune-tolerance mechanisms:

2.1. Normal trophoblasts express Human Leukocyte Antigen (HLA)-I molecules (HLA-G, -E, –C, and –F) but not HLA-II molecules, which helps GTDs avoid T-cell identification and cytolysis but leaves them vulnerable to natural killer (NK) cell cytotoxicity, as shown in Table 2. The trophoblast appears to be protected from cytolysis by NK cells, the main subset of immune cells in the uterine decidua, when nonclassical (E, G, F) and classical (C) HLA antigens are expressed.[39]
2.2. Lymphocytic activity is suppressed by choriocarcinoma which helps escape the host immune response, as evidenced by decreased expression of conventional activation markers (CD69 [NK cells], CD71 [macrophages], CD134 [T cells], CD3/HLA-DR [T cells]) and the apoptosis marker CD95 on those lymphocytes (Fas).[40]
2.3. Natural killer cells,[41] macrophages,[42] T cells,[43] and small numbers of Natural killer T cells[44] are present in uterine decidua

Table 2
List of antigens present on villi surface.

Antigens present on surface of villi
1. Human leukocyte antigen: The major classical HLA class Ia molecules HLA-A or HLA-B and HLA class II molecules are not expressed, even when stimulated with interferon (IFN)-c. Nonclassical class I HLA-G, HLA-E, HLA-F, the classical HLA-C, and CD1d (a ligand for natural killer T cells [NKT] cells that resembles HLA-I) are expressed on EVT.[21] HLA-C is a key molecule that can elicit allogeneic immune responses by maternal T and NK cells.[22] The membrane type of HLA-G, HLA-E, HLA-F on binding to respective ligands suppresses the cytolytic function of natural killer cells. Expression of the soluble form of HLA-G induces apoptosis of peripheral cytotoxic T lymphocytes (CTLs).[23]
2. Human endogenous retrovirus: Five HERVs have been found on trophoblasts (HERV-E, HERV-H, HERV-L, HERV-R [also called ERV3], and ERV9). ERVs are expressed mainly on placental syncytiotrophoblasts, not on EVTs.[24,25] Release of metalloproteinases, syncytiotrophoblast formation, immunosuppression activity in decidua, the formation of pseudotumor and metastatic properties to the trophoblast, are the biological roles of placental ERV. They also regulate the balance between proliferation, survival and apoptotic degeneration of placenta[26,27]
3. Fas–FasL system: Fas is highly expressed on activated T cells, B cells, NK cells, and macrophages. FasL is expressed on EVTs, villous cytotrophoblasts, and syncytiotrophoblasts. When trophoblast cells invade the uterine decidua, maternal immune tolerance is maintained by destruction of these cells by T cells due to the interaction of FasL on the trophoblast surface with Fas on activated T cells[28,29] The expression of Fas on the trophoblast is promoted by Type 1 T-helper cell (Th1; proinflammatory) cytokines, such as TNF and IFN-c,thereby priming them apoptosis, whereas the type 2 T-helper cell (Th2; anti-inflammatory) cytokines, such as interleukin (IL)-6 and IL-10, increase the expression of FasL and causing suppression to apoptosis[30]
4. Complement system: The activity of complement system in human innate immunity is regulated by three types of cell membrane proteins, which are all presented on human syncytiotrophoblasts, villous trophoblasts, and EVTs.[31] DAF (also known as CD55) and membrane cofactor protein (MCP, or CD46), both participate in controlling C3 activation at early stage of the complement cascade; CD59 acts at later stages to prevent formation of the lytic lesion generated by the membrane attack complex.[32]
5. Indoleamine 2,3-deoxygenase: Villous syncytiotrophoblasts, villous endothelial cells, and EVTs express the IDO, which is thought to induce maternal immunotolerance to the trophoblasts by both decreasing the activity of T cells and inhibiting the complement cascade reaction.[33,34,35]
6. Chemokines: CCR1, CCR10, CXCR1, and the CCR1 with their ligands regulated-upon-activation normalT-cell-expressed and secreted (RANTES) protein, MIP-1a, macrophage inflammatory protein (MCP-2), and Human CC chemokine 1 (HCC-1) have been detected on EVTs at the messenger RNAs (mRNAs) and protein levels, and patchy expression of MIP-1a has been detected on syncytiotrophoblasts.[36]
7. Cytokines: Placental Trophoblast and villous stromal cells secrete numerous cytokines, like IFNs (alpha, beta and gamma), ILs (IL-1alpha/beta, IL-6, IL-2, IL4, IL-10, IL-13, IL-15, IL-1RA), members of the transforming growth factor (TGF)–b superfamily (TGF-b1, TGF-b2, TGF-b3), growth factors (granulocyte and macrophage colony-stimulating factors [GM-CSFs]), and TNFa and b.[37] These cytokines regulate trophoblast growth, proliferation, and differentiation to prevent trophoblast overgrowth and invasion.[38]

during normal pregnancy. During a normal pregnancy, they can be found in the uterine decidua. In GTD, the maternal immune cells are distributed differently than in normal pregnancies. According to a study by Kabawat et al., complete hydatidiform moles (CHM) had a larger number of CD4 T cells and a higher CD4/CD8 T cell ratio.[45] Wongweragiat et al. found that the decidua of CHM includes a larger percentage of CD3 T cells and CD4 T cells than normal early pregnancy decidua. Interestingly, only the number of CD4 T cells in CHM was larger than in Partial hydatidiform mole. However, the CD8/CD4 T cell ratio in CHM (1.6:1) was considerably lower than in normal early pregnancy (3.2:1, $p = 0.02$) and PHM (2.4:1, $p = 0.03$).[46] However, choriocarcinoma and molar pregnancy have an increased number of CD8/CD3 T lymphocytes in decidua as compared with normal pregnancy.[47]

2.4. The expression of HCG receptors is linked to the tumor's invasive properties.[48] The expression of Fas L mRNA is elevated by HCG without affecting the expression of Fas.[49] In comparison to normal pregnancy, hydatidiform mole and choriocarcinoma have higher HCG receptor expression and HCG production.[50] This may cause greater immunosuppression at the primary uterine location, allowing malignant trophoblast to avoid detection by the maternal immune system.

3. Immunotherapy as treatment option

Immunotherapy is emerging as a ludicrous option in treatment of the chemorefractory, recurrent GTN. Traditionally most patients of GTN are treated with chemotherapy, either single agent or multi drug chemotherapy and it is seen to have 65%–95% successful remission rate. In up to 5% patients, the usual chemotherapeutic regimens fail and these cases are usually fatal. In such cases, there is a need for introduction of novel agents that can be used in their treatment.[51]

4. PD-1/PD-L1 inhibitors in patients with chemoresistant GTN (Table 3)

Paternal antigens expression on placenta and fetus make these organs a target for maternal immune system. During normal pregnancy course, T-cell proliferation is suppressed due to several mechanisms like programmed cell death 1 ligand 1 (PD-L1) which is present on trophoblasts when interact with PD 1 on T cells inhibits maternal T cell function and hence prevent fetal rejection.[52] PD-L1 is expressed in trophoblastic tissue of all types of GTN patients.[53] This could be the reason of a sluggish maternal antitumor immune response, which leads to trophoblast growth and GTN. Nonclassical class I HLA-G, HLA-C, and HLA-E are also expressed by trophoblasts.[54] These non-classical major histocompatibility complex (MHC) class I molecules interact with NK cells and prevent lysis of trophoblast cells.[55] PD1/PDL1 pathway plays an important role in T helper type 17 (Th17) and regulatory T cells (Tregs) regulation. Blockade of this pathway, results in diminished Treg function and increased Th17 cell population which causes excessive inflammatory response.[56] This mechanism can be used for an effective cancer immunotherapy. The anti-PD-L1 monoclonal antibody avelumab and anti-PD-1 drug pembrolizumab have been tried in chemotherapy resistant GTN patients. Avelumab has shown promising results in a phase II trial for chemotherapy resistant GTN patients with a favourable toxicity profile as compared to chemotherapy.[57] Pembrolizumab was tried in four patients with chemotherapy resistant GTN resulting in good efficacy and low toxicity in these patients (Tables 3 [58–61]).

Multiple case studies have also been reported that justify the use of PD1 inhibitors in GTN. Goldfarb st al,[62] reported a case of multi drug resistant choriocarcinoma with full thickness myometrial involvement and multiple pulmonary nodules. She was treated with six different chemotherapy regimens but her beta HCG levels continued to rise. She experienced remission of 24 months after treatment with Pembrolizumab.

Table 3
Trials supporting the use of PD1/PD L1 inhibitors in multidrug resistant metastatic gestational trophoblastic neoplasia.

Study	Patient characteristics/ regimen	Result	Inference
Ghorani et al.[51]	4 patients: 2 metastatic choriocarcinoma 2 metastatic ETT/ PSTT Resistant to multidrug chemotherapy	Choriocarcinoma 2 out of 2 patients show Partial response (PR) ETT/PSTT 1 patient has Partial response (PR) 1 patient has persistent disease (PD)	Promising results of pembrolizumab in drug resistant gestational trophoblastic neoplasia
Choi et al.[58]	15 patients Chemo-Resistant Gestational Trophoblastic neoplasias Pembrolizumab dose of 200 mg every 3 weeks Phase 2 trial commencing in June 2020	Results awaited	–
Choi et al.[59]	7 patients: 3 choriocarcinoma, 4 ETT/PSTT Resistant to numerous cytotoxic, single- or multi-agent, chemotherapies pembrolizumab 200 mg, every 3 weeks	Choriocarcinoma 3 out of 3 patients show Complete response (CR) ETT/PSTT 2 out of 4 patients show CR 1 patient has Partial response (PR) 1 patient has persistent disease (PD)	Treatment outcomes with pembrolizumab for refractory gestational trophoblastic neoplasia showed a favourable response rate of 85.7% (6/7) and a CR rate of 71.4% (5/7)
TROPHIMMUN trial[60]	29 patients of chemo resistant gestational trophoblastic neoplasia Avelumab administration at 10 mg/kg Phase 2 trial is currently in recruitment phase	Results due in 2023	–
CAP 01[61]	20 patients (19 choriocarcinoma and 1 placental site trophoblastic tumour.) 4-week cycles of intravenous camrelizumab 200 mg every 2 weeks plus oral apatinib 250 mg once per day	Objective response rate was 55%; 10 patients had complete response.	Camrelizumab plus apatinib showed promising antitumor activity and acceptable toxicity .

Another report by Huang et al.,[63] shows that a chemoresistant choriocarcinoma with metastasis to pancreatic head, liver and lungs when treated with Pembrolizumab demonstrated remarkable response after 2 cycles and near-complete resolution of all lesions on PET imaging.

In a study of 16 cases by Kong et al.,[64] 5 patients out of 16 relapsed; one patient from the relapsed group was started on pembrolizumab. According to Shih and Kurman's hypothesis,[65] it is surmised that chemosensitive choriocarcinoma tumor cells are destroyed by the use of multiple courses of chemotherapy and the remaining cells differentiated

into intermediate trophoblastic cells which do not respond to chemotherapy and here immune checkpoint inhibitors come into play.

Other newer treatment modalities: These treatment options are mentioned to highlight the immune mechanisms that are being under research and can be exploited to develop new treatment options for GTD. Treatment modalities like antibodies attached to direct enzyme prodrug therapy show a target against specific tumor antigens. Similarly Neural stem cells can be used in immunomodulation and anti VEGF monoclonal antibodies are being explored in treatment of GTD.

4.1. Direct enzyme prodrug therapy (DEPT): It is a type of therapy in which artificially induced enzymes are used to convert prodrugs to drugs at a desired location.[66] It is beneficial in the sense that drug related toxicity is significantly reduced as the active drug is present only at the desired location.[67] Taking advantage of this feature multiple studies are being conducted to see its response in patients with choriocarcinoma.

In Antibody-DEPT (ADEPT), antibodies are connected to enzymes which are targeted against specific cancer antigens. These enzymes can bind to distinct cancer cells. Their anti-tumor effect has been demonstrated in multiple choriocarcinoma animal xenograft models.[68]

In Gene-DEPT (GDEPT), selective genes are delivered to tumor sites which can convert prodrugs to drugs at specific sites.[69] Tumor-specific promoters or viral transfection selective expresses these genes at the tumor site. It was further confirmed by Weyel et al. that the tumor growth in the choriocarcinoma xenograft model was supressed by GDEPT using β-glucuronidase, which converts HMR 1826 to doxorubicin.[70]

4.2. Genetically engineered neural stem cells: Neural stem cells are self-renewing, multipotent cells that originate from radial glial progenitor cells which are used to generate neurons and glial cells of the nervous system.[71] They have unique features like proliferation, integrating with the host cells without affecting the tissue microenvironment and migration towards neoplastic tissue.[72] These properties can be used to target tumor cells. It is seen that the Neural stem cells (NSC) show tumor-tropism as they are attracted towards the hypoxic regions in the tumor.[73] It was seen in study on intracranial tumors that the hypoxic regions have increased expression of many chemoattractant molecules like vascular endothelial growth factor (VEGF)/VEGF-receptor, stem cell factor/c-kit system, hepatocyte growth factor/c-met signaling, Annexin II, high mobility group box 1/receptor for advanced glycation end products and urokinase-type plasminogen activator. These chemoattractant molecules facilitate the migration of NSCs towards the tumor.[74] Due to this property, NSCs containing the anticancer gene can aid in the treatment of metastatic GTD.

4.3. Combining GDEPT with NSC: Genetically modified NSCs can be combined with GDEPT, where it can be used for carrying a gene that can code for an enzyme which can convert a prodrug to active cytotoxic metabolite. Since the gene connected to the NSC promotes selective migration of the stem cells toward the tumour and hence activation of the drug at the specified tumour site, the aforesaid technique can be cancer specific with less risk of adverse drug reactions and higher therapeutic efficacy.[75]

Further NSCs can be engineered to express immunomodulatory genes, responsible for the expression of interferon (IFN)-β, IL-4, IL-12 and IL-23, and they can be used to effectively treat cancer by expressing them at the tumor site.[76] Treatment with these stem cells in choriocarcinoma metastasis or xenograft models inhibited tumor growth and decreased metastasis as quoted by Kim et al.[77]

4.4. Anti VEGF monoclonal antibody - Endocrine gland-derived vascular endothelial growth factor (EG-VEGF) is a specific placental angiogenic factor. Balance between the trophoblast and endothelial cells is important for development of normal placenta. Disruption in the expression of angiogenic factors and poor placental vascularization during the first trimester of pregnancy is found to be associated with development of GTN.[78] Monoclonal antibody to VEGF like Bevacizumab and monoclonal antibody to endoglin (CD105) likeTRC 105 (carotuximab) have been tried in refractory GTN cases.[79]

5. Conclusion

To summarize, gestational trophoblastic diseases are a group of tumors linked to pregnancy, and their immunobiology reveals that they can trigger a strong rejection reaction in the mother's body, which is suppressed by GTD's immunity-evading mechanism. Many studies have demonstrated that during a normal pregnancy, the maternal immune system can control trophoblast development, proliferation, migration, and invasion. GTN is caused by the maternal immune system's vulnerability, as well as the trophoblasts' heteromorphic genetic background. To completely comprehend the immunobiology of these tumors, more research into the intricacies of the immunological processes underlying both normal trophoblasts and GTD is required. Immunotherapy, which takes advantage of maternal immune components to treat patients with persistent and chemoresistant GTDs is quickly becoming a must-have treatment option.

Author contributions

NS made a substantial contribution to the concept or design of the article; NS was responsible for the acquisition, analysis, or interpretation of data for the article, and Drafted the article or revised it critically for important intellectual content. RK helped in concept of design and drafted the article for important intellectual content. VK approved the version to be published.

Declaration of competing interest

The authors declare no conflicts of interest.

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