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Decidual Immune Cell Infiltrate in Hydatidiform Mole

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ABSTRACT

Background: **Hydatidiform mole is a gestational trophoblastic disease characterized by proliferation of the pregnancy-associated trophoblastic tissue. Complete hydatidiform mole is an entirely paternally derived lesion, and therefore, represents complete intrauterine allografts that can induce an altered maternal immune response** ***Hypothesis:*** **Here, we hypothesize that "the development of hydatidiform moles is associated with numeric alterations of the decidual immune cell infiltrate."** ***Materials and methods:*** **A total of 30 specimens (decidual tissue), entailing normal first trimester pregnancy terminations and complete hydatidiform moles (15 cases, each), were evaluated for immune cell infiltrate using immunohistological methods and monoclonal antibodies (CD20, CD68, and CD3 for B cells, histiocytes/dendritic cells, and T cells, respectively).** ***Results:*** **Groups of immune cells were seen in the decidual tissue of first trimester normal pregnancy terminations and hydatidiform moles. Compared to the decidual tissue of first trimester normal pregnancy terminations, the mean counts of the immune cells were statistically significantly higher ($p < 0.05$) in the decidual tissue of the hydatidiform moles (0.33 ± 0.21 vs. 1.66 ± 0.21 for $CD20^+$B cells; 9.80 ± 1.57 vs. 13.14 ± 1.16 for $CD68^+$ cells; and 12.92 ± 3.46 vs. 23.85 ± 1.22 for $CD3^+$cells for decidual tissue without and with molar changes, respectively).** ***Conclusions*****: Hydatidiform moles are associated with numeric alterations of immune cell infiltrate. The numeric dominance of immune cells in the hydatidiform moles may reflect either non-specific or specific immunological processes. The possible pathogenetic and prognostic ramifications of our findings are open for further investigations.**

INTRODUCTION

The gestational trophoblastic diseases are characterized by proliferation of the pregnancy associated trophoblastic tissues. These lesions include hydatidiform moles, invasive moles, exaggerated placental sites, placental-site nodules, placental-site trophoblastic tumors and choriocarcinomas. Hydatidiform mole represents abnormal placenta with marked enlargement of the chorionic villi due to cystic, hydropic swelling of the tissues that is associated with hyperplastic and anaplastic changes in the chorionic epithelium. Hydatidiform mole is a common complication of pregnancy, occurring about once in every 1000–2000 pregnancies. Most patients with hydatidiform moles present with vaginal bleeding. They also present with a uterus that is usually larger than the expected for the duration of pregnancy. Also, the serum human chorionic gonadotrophins levels increase more rapidly in hydatidiform moles compared to normal pregnancy (1, 2).

In normal pregnancy, around 30% of the stromal cells in decidualized endometrium are leukocytes (3, 4). Of these, up to 70% are phenotypically unusual endometrial granulated lymphocytes. The T lymphocytes and macrophages constitute approximately 10–20% and 20% of first trimester decidual leukocytes, respectively. These immune cells play critical roles in intrauterine immunoregulation and cytokine production (2, 4,

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5). Complete hydatidiform moles are totally paternally derived and, therefore, represent complete allografts that may stimulate maternal immune rejection (6, 7). This notion is supported by several observations. Leukocytes cell infiltrate is a constant feature in the hydatidiform moles indicating ongoing immune reaction to unidentified antigens, possibly as part of the allograft rejection (6, 8). Also, it is well known that macromolecules derived from the hydatidiform moles could interfere with in the vitro immunologic response by modulating interleukin-2 function (9). In addition, the extracts from the trophoblast of hydatidiform moles can suppress interleukin-2-induced proliferation of human T-lymphocytes and phytohemagglutinin-blasts (10). This trophoblast produces a beta-interferon-like macromolecule that can abrogate the maternal rejection response harmful to the developing fetal allograft (11).

The immune defense against allograft is largely a cell-mediated one that relies on the integrity of T lymphocytes ($CD3^+$ cells) and the antigen presenting cells ($CD68^+$ cells). Moreover, B cells ($CD20^+$ cells) can mount humoral immune response. Moreover, a great deal of work has established that the cellular ($CD3^+$ T cells) and the humoral ($CD20^+$ cells) branches of the immune system as well as the antigen presenting cells ($CD68^+$ cells) are relevant to human tumor immunology (12–15). As techniques for isolating and identifying cells associated with immune responses developed, it became clear that these cells are critical for immune responses. Thus, understanding the status of these cells in the trophoblastic tumors will largely come to mean understanding the development of these lesions. In hydatidiform moles, alteration of immune cells (lymphocytes, monocytes, histiocytes, and dendritic cells) seems to have a role in the neoplastic process. In support, Knoeller et al. observed a significantly increased number of lymphocytes positive for CD8, CD3 in choriocarcinomas and hydatidiform moles compared to the samples from normal pregnancy (6). Wongweragiat et al. found that the numbers and percentages of $CD3^+$ and $CD4^+$ T cells were significantly increased in complete hydatidiform moles compared to partial moles and normal early pregnancy decidua (4, 5).

To date, immunophenotype characterization of immune cell infiltrates in the hydatidiform moles is incomplete. This study investigates those characteristics to test the hypothesis that "the development of hydatidiform moles is associated with numeric alterations in the decidual immune cell infiltrate." To achieve this goal, immune cell infiltrates from patients with normal first trimester pregnancy terminations and hydatidiform moles (15 cases, each) were evaluated using immunohistochemical methods and monoclonal antibodies.

MATERIALS AND METHODS

Patients

This controlled prospective study included 30 patients, divided into two groups. The first group consisted of 15 patients with complete hydatidiform moles (diploid moles, with 46 XX karyotype). The second group included 15 patients with early first trimester pregnancy terminations (between 9 and 12 weeks of gestation). The study was performed in the Hospital of Ibn Sina College of Medicine, Jeddah, Saudi Arabia. Informed consents were obtained from all the participants. The incidence of the hydatidiform moles based on the ethnicity and the geographic distribution was 7 cases per 100,000 (Saudi females, Jeddah region). The incidence of the hydatidiform moles was 1 case per 100,000 (non-Saudi females, Jeddah region). The diagnosis of early pregnancy failure was established based on the findings of the transvaginal ultrasonography scans. It was defined as the presence of an intrauterine gestational sac (sac diameter $>$ 15 mm or $<$15 mm not showing any growth after a 7 days interval) with or without an embryonic pole and with absence of cardiac activity. The diagnosis was confirmed by histopathological examination of the tissue specimens (products of conception). The diagnosis of complete hydatidiform moles was based on the findings of the pelvic ultrasound scans and the quantitative estimation of serum beta human chorionic gonadotrophin. The diagnosis was established based on the histopathological examination of the tissue specimens (products of conception with molar changes). Most of the patients admitted with complete hydatidiform moles were initially diagnosed as threatened abortion. Seventy-six percent of the patients in the study groups were non-Saudi. The details of maternal characteristics, clinical presentations, management and complications of the conditions were noted from the case records. Patients with molar pregnancy were followed up for a period ranging from 8 months to 2 years after treatment. A quantitative serum beta human chorionic gonadotrophin was carried out.

Tissue specimens

The study included 30 specimens obtained from patients with normal first trimester pregnancy terminations and hydatidiform moles (15 cases each). Formalin fixed, paraffin embedded tissues was obtained from the Department of Pathology, Ibn Sina National College of medicine, Jeddah. All tissue blocks were sectioned at 3.0 μm and mounted on 3-aminopropyltriethoxysilane coated slides. At least ten sections from each tissue block were stained with haematoxylin and eosin for histological evaluation.

Histological evaluation of the immune cells

Histologically, hydatidiform moles showed hydropic changes of the chorionic villi, absence or inadequate development of vascularization of the villi and the proliferation of villous trophoblastic cells. The proliferating trophoblastic elements were composed of syncytiotrophoblast, cytotrophoblast, and intermediate trophoblast. The trophoblastic cells showed considerable cytologic atypism with nuclear and cytoplasmic enlargement, irregularity of nuclear outlines, and hyperchromasim. The central substance of the villi was composed of a loose, myxomatous, edematous stroma with central cisterns (1). Cases which showed histological evidence of decidual necrosis or inflammation identified by the presence of neutrophil polymorphs or plasma cells were not included in the study. The cells (lymphocytes and histiocytes) were evaluated following other groups (14, 16).

Table 1. Summary of the antibody dilutions and retrieval solutions

Antibodies	Specificity	Retrieval solution	Dilution	Incubation time	Control tissue (Lymph node)	Distribution of staining
CD68	Histiocytes Dendritic cells	Citrate, pH = 6	1:100	30 min at 37°C	Sinusoidal	Cytoplasmic, granular
CD3	Pan T cells	Trypsin	1:100	30 min at 37°C	Paracortical	Membranous
CD20	Pan B cells	Citrate	1:100	30 min at 37°C	Follicular	Membranous

Antibody selection

A panel of three mouse monoclonal, Anti-Human antibodies decorating B lymphocytes (CD20), T lymphocytes (CD3) and histiocytes/dendritic cells (CD68) was used to phenotype the immune cells. The rationales behind this selection include: i) lymphocytes and macrophages represent 10 and 20% of first trimester decidual leukocytes, respectively, and ii) the immune defense against neoplastic proliferation and allograft is basically a cell-mediated one that that involves T lymphocytes ($CD3^+$ cells) and the antigen presenting cells ($CD68^+$ cells). Moreover, B cells ($CD20^+$ cells) can mount humoral immune response that plays some roles in these conditions (4, 5, 17).

We used Anti-Human CD20cy antibodies to examine B-lymphocytes. This antibody labels cells of the B- cell lineage. CD20 is a transmembrane, non-glycosylated protein found on B-cell precursors and mature B cells but is lost following differentiation to plasma cells (18, 19). To immunophenotype T cells, we used Anti-Human CD3 monoclonal mouse antibody. This antibody labels CD3ε-chain and is a useful tool for the identification of T cells in man and various animals. The CD3 antibody recognizes an epitope on the intracytoplasmic portion of the ε-chain of CD3 (20, 21). The monoclonal mouse Anti-Human CD68 antibody was used to decorate histiocytes/dendritic cells. This antibody labels macrophages and other members of the mononuclear phagocytic lineage. CD68 is a highly glycosylated lysosomal membrane protein. It is expressed strongly in the cytoplasmic granules of the monocytes (22, 23). A summary of the staining conditions and the characteristics of the antibodies used in this study are presented in Table 1.

Immunohistochemical analysis of the immune cells

Immunostaining was carried out as previously described by Hussein et al. (12, 16). Sections mounted on glass slides were deparaffinized and rehydrated. The endogenous peroxidase activity was blocked. Non-specific protein binding was blocked, and the sections were then incubated with mouse monoclonal antibodies for 30 min at room temperature [(B lymphocytes: Anti-Human CD20cy, Clone L26, Code-Nr.M0755); (T lymphocytes: Anti-Human CD3, Clone PC3/188A, Code-Nr.M7193) and (Histiocytes/dendritic cells: CD68, Clone KP1, Code-Nr.M0814); DakoCytomation Denamark A/S-DK-2600 Glostrup]. A secondary-staining system (LSAB2, DakoCytomation Denamark A/S-DK-2600 Glostrup) was used according to the manufacturer instructions. Sections were next treated with Peroxidase-labeled Streptavidin for 30 min at room temperature and incubated with 14-diaminobenzidine and 0.06% H_20_2 for 5 min. The sections were counterstained with hematoxylin, dehydrated in alcohol, cleared in xylene and cover-slipped. The slides were independently evaluated by the authors. The counts of the immune cells were combined to give a final figure. The slides were patch stained (tissue from the hydatidiform moles and the normal first trimester pregnancy terminations were stained in one run for each antibody). The immunostaining experiments were repeated three times to get consistent results (12, 13).

Positive controls

Specimens consisted of lymph nodes with reactive lymphoid hyperplasia (Germinal centers, paracortex and tangible body macrophages for CD20, CD3 and CD68, respectively) served as positive controls (12, 13).

Negative controls

Additional sections, running in parallel but with omission of the primary antibody served as the negative controls (12, 13).

Evaluation of the immunostaining

Corresponding sections stained with hematoxylin and eosin were examined side by side with the immunostained sections. In each case, entire section was histologically examined by bright field microscope at low power magnification to detect sites of antibody positivity and then higher power magnification was used to evaluate immunostaining. Sections were examined independently by the authors. The positive staining was evaluated by counting 100 cells in the areas of the highest expression. CD3 and CD20 positive signals were identified as membranous brown rim around the basophilic nucleus. Signals for CD68 appeared as diffuse and granular cytoplasmic signals (12, 13). The results were expressed as mean $\pm$ standard error of mean (SEM) (4, 16).

Statistical analysis

Results were statistically analyzed and computed on IBM PC microprocessor using statistical package for Social Sciences SPSS for Windows. Fisher Exact Test and ANOVA (Analysis of Variance) were used. A nominal significance level of $p < 0.05$ was used.

RESULTS

Clinical features

The clinical presentations of the molar pregnancies included vaginal bleeding, hyperemesis gravidarum and the disparity

between the uterine size and the period of gestation. Patients with hydatidiform moles were followed up by the estimation of serum beta human chorionic gonadotrophin levels and regular ultrasonography scans. The patients were advised to avoid pregnancy until beta human chorionic gonadotrophin levels became normal and persist as such for at least 6 months. Combined oral contraceptive pills were prescribed to the patients as the method of birth control. The data characteristics of the patients are summarized in Table 2.

Immunohistological features of immune cells in the normal first trimester pregnancy terminations and hydatidiform moles

Lymphocytes and histiocytes were seen infiltrating the decidual tissue of the normal first trimester pregnancy terminations and hydatidiform moles. The density of these cells was high in the molar decidual tissue compared to the decidua of the normal first trimester pregnancy terminations (Fig. 1). Immunohistochemical evaluations showed that the positive and negative controls were positive and negative, respectively, indicating the validity of our immunostaining results. In the normal first trimester pregnancy decidual tissue, most of the immune cells were $CD3^{+}$T and $CD68^{+}$ cells. However, rare CD20 B lymphocytes were also seen. In complete hydatidiform moles, the number of the decidual $CD3^{+}$T lymphocytes and CD68 $^{+}$ cells

Table 2. Data characteristics of the patients with the normal first trimester pregnancy terminations and hydatidiform moles

Clinical features	Normal first trimester pregnancy terminations n = 15 cases	Hydatidiform moles n = 15 cases
Age (years)		
≥ 20	3/15 (20%)	1/15 (6%)
21–35	10/15 (66%)	12/15 (80%)
≥ 35	2/15(13%)	2/15 (13%)
Parity		
0–5	6/15 (40%)	5/15 (33%)
≥ 3	9/15 (60%)	10/15 (66%)
Presenting features		
Vaginal bleeding	15/15 (100%)	10/15 (66%)
Hyperemesis gravidarum	1/15 (6%)	9/15 (60%)
Anemia	6/15 (40%)	8/15 (53%)
Pregnancy associated hypertension	1/15 (6%)	4/15 (26%)
Pregnancy associated thyrotoxicosis	0/15 (0%)	1/15 (6%)
Uterine size		
Larger than the expected date	0/15 (0%)	7/15 (46%)
Smaller than the expected date	10/15 (66%)	4/15 (26%)
Compatible with the expected date	5/15 (33%)	4/15(26%)
Ovarian enlargement	0/15 (0%)	6/15 (40%)

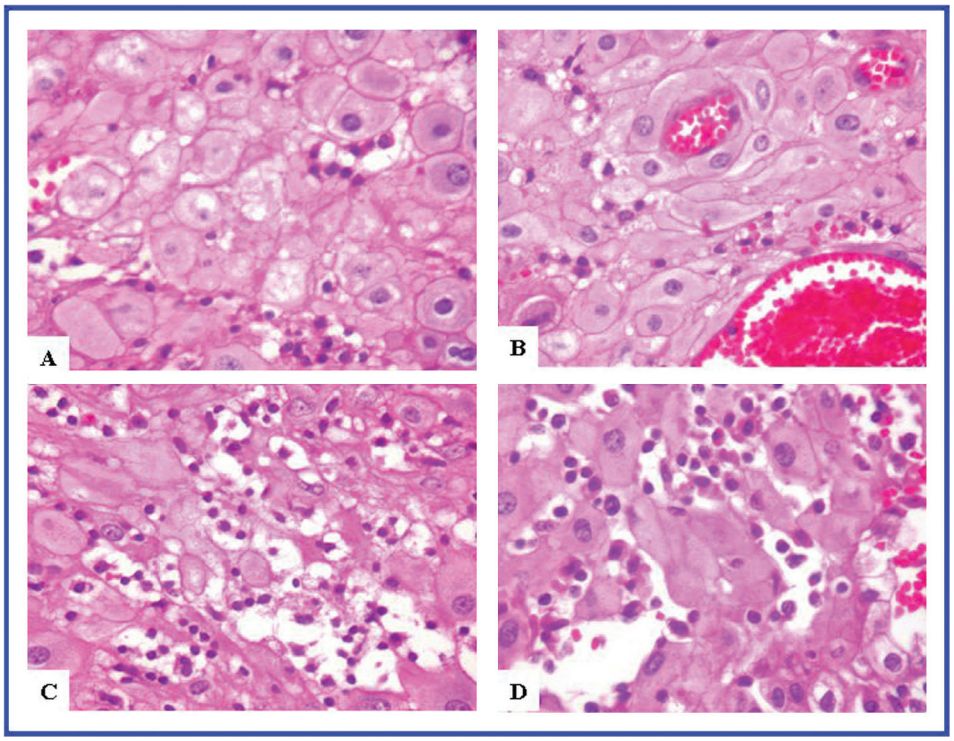

Figure 1. Cellular infiltrate in normal first trimester pregnancy terminations and hydatidiform moles. Cellular infiltrate in normal first trimester pregnancy terminations (A–B) and hydatidiform moles (C–D). Lymphocytes, histiocytes are seen infiltrating the decidual tissue of the normal first trimester pregnancy terminations and hydatidiform moles. The density of these cells is high in the molar decidual tissue (C–D) compared to normal first trimester pregnancy terminations (A–B).

were statistically significantly high ($p < 0.05$) compared to normal first trimester pregnancy decidua. A summary of these findings is shown in Table 3 and Fig. 2.

Table 3. Mean counts of $CD68^+$, $CD20^+$ and $CD3^+$positively stained cells in the normal first trimester pregnancy terminations and hydatidiform moles

Aspects	Normal first trimester pregnancy terminations n = 15 cases	Hydatidiform moles n = 15 cases
$CD20^+$B lymphocytes		
Mean	0.33 ± 0.21	1.66 ± 0.21
Median	0.0	2.0
Range	0.0–1.0	1.0–2.0
$CD3^+$T lymphocytes		
Mean	12.92 ± 3.46	23.85 ± 1.22
Median	20	23
Range	0.0–30	20–30
$CD68^+$cells		
Mean	9.80 ± 1.57	13.14 ± 1.16
Median	14.0	15.0
Range	3.0–17.0	10–17

DISCUSSION

Hydatidiform mole represents an abnormal placenta characterized by excessive enlargement of chorionic villi caused by central edema of the stroma and variable hyperplasia of the villous trophoblastic cells. Since all the chromosomes are paternal in origin, a complete hydatidiform mole is an intrauterine allograft within the mother and may therefore be able to induce maternal immune responses leading to fetal rejection and recruitment of immune cells to the decidual tissue. The present study was carried out to examine the numeric alterations of the decidual immune cells in the complete hydatidiform moles compared to the normal first trimester pregnancy terminations. Our investigation clearly demonstrates a numeric dominance of immune cells in the decidual tissue of hyaditiform mole relative to normal first trimester pregnancy terminations. It is unlikely that this numeric dominance reflects decidual inflammation for two reasons. $CD20^+$ B cells did not achieve numeric dominance in the molar pregnancy decidua. In addition, none of the specimens showed evidence of decidual necrosis, acute inflammatory cells, or plasma cell infiltrates.

In our series, the presence of decidual immune cells in normal first trimester pregnancy terminations is in agreement with previous studies (2–7). T cells and macrophages were the predominant cell populations. Decidual T cells express various activation

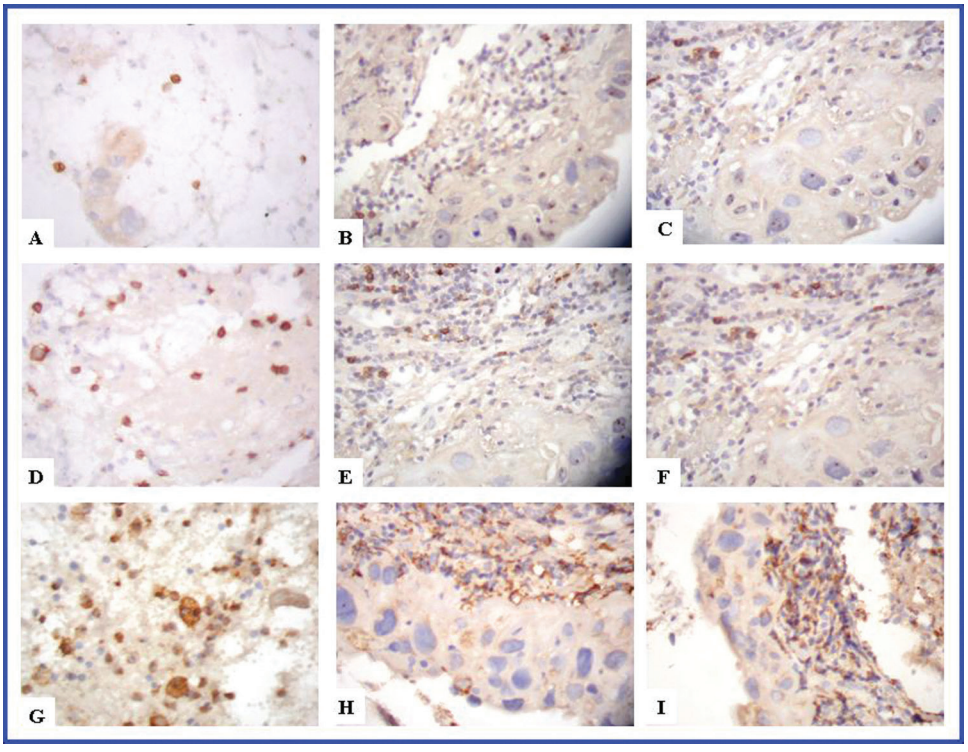

Figure 2. Immunohistological features of immune cells in the normal first trimester pregnancy terminations and hydatidiform moles. Staining of the immune cells in the normal first trimester pregnancy terminations (A: CD20; D: CD3 and G: CD68) and in hydatidiform moles (B–C: CD20; E-F: CD3 and H-I: CD68). Most of the immune cells in the decidual tissues of the normal first trimester pregnancy terminations and hydatidiform moles are $CD3^+$T and $CD68^+$ cells. Rare $CD20^+$ B lymphocytes are also seen. However, in hydatidiform moles, the density of $CD3^+$T lymphocytes and $CD68^+$ cells are high compared to normal early pregnancy decidua.

markers such as HLA-DR, CD69, and interleukin (IL)-2 receptor. Th2-type cytokines seem to be critical for successful pregnancy whereas Th1 type cytokines may be deleterious. T cell clones from early pregnancy decidua have defective production of Th2 type cytokines in unexplained recurrent abortions compared to normal first trimester pregnancy. Taken together, it is possible that there is a link among abnormal trophoblast proliferation, altered decidual leukocyte immune cells and local cytokine milieu. Functional studies of these issues mandate further investigations (2–7).

The presence of immune cell infiltrate in the decidual tissue of hydatidiform moles not only concurs with previous reports (2, 4–7) but also reflects ongoing immune responses against the trophoblastic cells. As the complete hydatidiform mole is androgenetic, it is possible that molar trophoblast may stimulate an altered maternal immune response with recruitment of immune cells. In our series, $CD3^+$ T cells achieved numeric dominance that may be due to increased recruitment, local proliferation of these cells or an increased turnover rate of these T cells in response to the molar trophoblast (4–6).

It is still possible that the marked increase in the density of immunecells in hydatidiform moles compared to spontaneous abortion may be due to increase in the load of associated antigens on these genetically altered trophoblastic cells. The extravillous trophoblast in normal pregnancy, molar trophoblast and choriocarcinoma cell lines expresses a non-classical class I MHC antigen, HLA-G (24, 25). Circulating immune complex with antigenic component composed of paternal HLA-G antigens were found in hydatidiform moles (25, 26). These antigens show some characteristics of Qa murine antigens mapped in the HLA system linked to class I antigens (24, 25, 27). Moreover, the molar trophoblastic cells may produce more cytokines than the normal pregnancy decidua that recruit more immunocytes. It is possible therefore that the invasive trophoblast within decidua leads to activation of decidual T cells. T cells in normal pregnancy may contribute to local cytokine production. The increased density of T cells, and the counts of macrophages may result in altered cytokine production, which can enhance either cell-mediated cytotoxicity or humoral immunity in response to molar trophoblast. The presence of few $CD20^+$ B cells in the present studies is in contrast with previous studies (4). It also suggests that humoral immunity is not the primary immune response mechanism.

To summarize, here, we report an increase in the density of decidual immune cell infiltrate in hydatidiform moles compared to the normal first trimester pregnancy terminations. The immune cell infiltrates included $CD20^+$, $CD68^+$, and $CD3^+$ cells. The $CD68^+$ and $CD3^+$cells were the predominant cell population indicating increased $CD3^+$ and $CD68^+$ cell proliferation and recruitment. The presence of both B and T lymphocytes in the gestational neoplastic lesions suggests the involvement of both cell mediated and humoral immunity in trophoblastic proliferation. It would be interesting to analyze some more functional elements like degree of apoptosis or the underlying mechanism (e.g., HLA expression).

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