Decidual T lymphocyte activation in hydatidiform mole

Sutatip Wongweragiat, Roger F Searle, Judith N Bulmer

Abstract

Aim—To quantify and compare decidual leucocyte subpopulations in complete and partial hydatidiform molar pregnancy with those in normal early pregnancy.

Methods—Decidual leucocytes were characterised using an avidin–biotin technique and a panel of monoclonal antibodies in formalin fixed, paraffin embedded decidual tissues from 10 normal first trimester pregnancy terminations and from 13 partial and 13 complete hydatidiform moles. Immunostained cells were fully quantitated and differences between subject groups were analysed using the Mann–Whitney test. T lymphocyte populations were further characterised using double immunohistochemical labelling.

Results—The numbers and percentages of CD3+ and CD4+ T cells were significantly increased in complete hydatidiform mole compared with partial mole and normal early pregnancy decidua. No differences were found in the number or percentage of CD8+ T cells. The CD8+ to CD4+ T cell ratio decreased significantly in complete mole compared with partial mole and normal decidua. The numbers and percentages of CD45RO+ cells increased significantly in both partial and complete hydatidiform mole compared with normal early pregnancy decidua. No differences were found in the number or percentage of CD8+ T cells. The CD8+ to CD4+ T cell ratio decreased significantly in complete mole compared with partial mole and normal decidua. The numbers and percentages of CD45RO+ cells increased significantly in both partial and complete hydatidiform mole compared with normal first trimester decidua. Double labelling confirmed that 50% of CD3+ T cells in complete and partial molar pregnancy coexpressed CD45RO, compared with 30% in normal pregnancy. Other leucocyte populations (eGLs, macrophages, B cells, and classical natural killer cells) did not differ between complete and partial mole and normal pregnancy decidua.

Conclusions—The presence of an increased population of activated decidual CD45RO+ T cells in complete and partial molar pregnancy suggests altered maternal immune responses against molar trophoblast.

(J Clin Pathol 1999;52:888–894)

Keywords: decidua; T lymphocyte; hydatidiform mole

In normal pregnancy around 30% of stromal cells in decidualised endometrium are leucocytes. Of these, up to 70% are phenotypically unusual endometrial granulated lymphocytes (eGLs); eGLs display natural killer (NK) cell cytotoxicity34 and produce various cytokines,5,6 but their in vivo function remains uncertain. T lymphocytes comprise approximately 10–20% of decidual leucocytes with a CD8+ to CD4+ ratio of around 3:1. These cells are also a potential source of cytokines in uteroplacental tissues. It has been proposed that a local T helper (Th) 2-type cytokine response favours fetal survival and successful pregnancy, whereas a Th1-type cytokine response may be detrimental.11 Macrophages constitute approximately 20% of first trimester decidual leucocytes and may play a role in intrauterine immunoregulation and cytokine production.12,14 It has been suggested that cytokines produced locally by different leucocyte populations at the uteroplacental interface regulate normal trophoblast proliferation and invasion, although the precise mechanisms are unknown.11,15–18

Hydatidiform mole is a gestational trophoblastic disease characterised by abnormal trophoblast proliferation. The incidence of molar pregnancy varies widely from 1/85 pregnancies in Indonesia19 to 0.6–1/1000 pregnancies in North America and Europe.20 Complete hydatidiform moles are usually diploid, with a 46,XX karyotype, and are of androgenetic origin, the majority resulting from the fertilisation of an anuclear ovum by a haploid sperm which then duplicates its own chromosome.21,22 In contrast, partial hydatidiform moles are generally triploid, resulting from the fertilisation of a normal ovum by two spermatozoa.23 Both partial and, more commonly, complete hydatidiform moles are associated with persistent trophoblastic disease. Since all the chromosomes are paternal in origin, a complete hydatidiform mole is an intrauterine allograft within the mother and may therefore be expected to stimulate maternal immune responses leading to fetal rejection. To date, there have been few and limited studies of maternal uterine immune responses in molar pregnancy.24–26 The present study was designed to characterise, quantify, and compare decidual leucocyte subpopulations and particularly T lymphocytes in complete and partial molar pregnancy with those in normal pregnancy to serve as a basis for the analysis of cytokine profiles in molar pregnancy. As well as analysing T lymphocyte subsets, expression of the CD45 isoforms CD45RA and CD45RO were analysed. CD45RA is expressed by naive or virgin T cells, whereas activated and memory T cells express CD45RO.27

Methods

Tissues

Formalin fixed, paraffin embedded decidual tissues from 10 normal first trimester pregnancy terminations and from 12 partial and eight complete hydatidiform moles were re-
trived from archive files of the department of pathology, Royal Victoria Infirmary, Newcastle upon Tyne. One partial and five complete hydatidiform moles were retrieved from archive files of the department of obstetrics and gynaecology, Siriraj Hospital, Mahidol University, Bangkok. All tissue blocks were sectioned at 3 µm and mounted on 3-aminopropyltriethoxysilane (APES; Sigma) coated slides. At least one section of each tissue was stained with haematoxylin and eosin (H&E) to allow morphological assessment. 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.

### Table 1 Primary monoclonal antibodies used on paraffin embedded sections

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Source</th>
<th>Specificity</th>
<th>Dilution</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3 (NCL-CD3-PS1)</td>
<td>Novocastra†</td>
<td>T cells</td>
<td>1:100</td>
<td>Pressure cooker (citrate buffer, pH 6.0)</td>
</tr>
<tr>
<td>CD4 (NCL-CD4-1F6)</td>
<td>Novocastra</td>
<td>Helper T cells, macrophages</td>
<td>1:50</td>
<td>Pressure cooker (EDTA buffer, pH 8.0)</td>
</tr>
<tr>
<td>CD8 (NCL-CD8-295)</td>
<td>Novocastra</td>
<td>Cytotoxic/suppressor T cells, NK cells, thymocytes</td>
<td>1:2000</td>
<td>Pressure cooker (EDTA buffer, pH 8.0)</td>
</tr>
<tr>
<td>CD20 (L26)</td>
<td>Dako‡</td>
<td>B cells</td>
<td>1:400</td>
<td>Pressure cooker (citrate buffer, pH 6.0)</td>
</tr>
<tr>
<td>CD45 (NCL-LCA-RP)</td>
<td>Novocastra</td>
<td>Lymphocytes, monocytes, eosinophils</td>
<td>1:100</td>
<td>Trypsin 5 min</td>
</tr>
<tr>
<td>CD45 RA (NCL-MB1)</td>
<td>Novocastra</td>
<td>B cells, monocytes and a small proportion of T cells</td>
<td>1:60</td>
<td>No pretreatment</td>
</tr>
<tr>
<td>CD45 RO (NCL-UCHL1)</td>
<td>Novocastra</td>
<td>Reactive T cells, monocytes, macrophages</td>
<td>1:400</td>
<td>Pressure cooker (citrate buffer, pH 6.0)</td>
</tr>
<tr>
<td>CD56 (NCL-CD68)</td>
<td>Novocastra</td>
<td>Macrophages</td>
<td>1:500</td>
<td>Pressure cooker (citrate buffer, pH 6.0)</td>
</tr>
<tr>
<td>CD57 (NCL-NK1)</td>
<td>Novocastra</td>
<td>NK cells</td>
<td>1:10</td>
<td>Trypsin 10 min</td>
</tr>
<tr>
<td>CD68 (NCL-CD68)</td>
<td>Novocastra</td>
<td>Macrophages</td>
<td>1:50</td>
<td>Trypsin 10 min</td>
</tr>
<tr>
<td>CD79a (CD79 ; JCB 117)</td>
<td>Dako</td>
<td>B cells</td>
<td>1:50</td>
<td>Pressure cooker (citrate buffer, pH 6.0)</td>
</tr>
<tr>
<td>Cytokeratin (8/18) (NCL-5D3)</td>
<td>Novocastra</td>
<td>Simple and glandular epithelium</td>
<td>1:20</td>
<td>Trypsin 10 min</td>
</tr>
<tr>
<td>CD34 (QBEND10, NCL-END)</td>
<td>Novocastra</td>
<td>Haematopoietic progenitor cells, vascular endothelium</td>
<td>1:25</td>
<td>Trypsin 10 min</td>
</tr>
</tbody>
</table>

†Novocastra Laboratories, Newcastle upon Tyne, UK.  ‡Dako, High Wycombe, UK.

### Antibodies

A panel of 11 murine monoclonal antibodies was employed to characterise leucocyte sub-sets. Antibodies directed against CD34 (QBEnd10) and cytokeratin 8/18 (5D3) were used to identify endothelial and trophoblast cells, respectively. All antibody specificities, dilutions, and pretreatments are detailed in table 1.

### Immunohistochemistry

#### Single labelling

Sections were labelled using a streptavidin–biotin complex immunohistochemical technique with no pretreatment, trypsin pretreatment, or pressure cooking in citrate buffer, pH 6.0, or EDTA buffer, pH 8.0. For each antibody the optimal dilution, incubation time, and pretreatment were established using similarly fixed and processed positive control tonsil and neuroblastoma tissues, as appropriate.

Sections were deparaffinised and rehydrated before quenching of endogenous peroxidase activity by incubation for 10 minutes with 1.6% hydrogen peroxide in methanol. For trypsinisation sections were incubated with 2.5% trypsin (Difco Laboratories) in distilled water, pH 7.8, containing 2.5% calcium chloride for five or 10 minutes. For pressure cooking sections were heated for one minute in citrate buffer, pH 6.0, or EDTA buffer, pH 8.0, as appropriate. For anti-CD4, CD8, and CD45RO, pretreatment by pressure cooking was followed by the use of an avidin/biotin blocking kit (Vector Laboratories) to block endogenous avidin and biotin activity. All sections were then overlain with 10% normal rabbit serum (NRS) in 0.1 M Tris/0.05 M saline (TBS), pH 7.6, for 10 minutes and then incubated with the primary antibody appropriately diluted in NRS for 60 minutes or overnight (anti-CD45). After washing twice in TBS, sections were incubated with biotinylated rabbit antimouse immunoglobulins (Dako), diluted 1:500 in NRS, for 30 minutes followed by streptavidin–biotin peroxidase complex (1:100 in NRS, Dako) for 30 minutes. The reaction was developed with 3,3’-diaminobenzidine (DAB; Sigma) containing 0.02% hydrogen peroxide for five minutes. Sections were lightly counterstained with Mayer’s haematoxylin, dehydrated, cleared in xylene, and mounted in DPX synthetic resin (Raymond A Lamb Co). Tonsil sections were used as a posi-
Table 2 Decidual leucocyte counts in normal first trimester pregnancy and in partial and complete hydatidiform moles. Each bar represents the percentage of the CD45 (LCA) population. Error bars = SEM. *p<0.05; **p<0.01; ***p<0.001.

<table>
<thead>
<tr>
<th>Decidual leucocyte populations</th>
<th>Normal pregnancy</th>
<th>Partial hydatidiform mole</th>
<th>Complete hydatidiform mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45 (LCA)</td>
<td>47 (37.5 to 73.3)</td>
<td>53.5 (29.6 to 80.4)</td>
<td>42.7 (27.4 to 86.0)</td>
</tr>
<tr>
<td>CD3</td>
<td>11.9 (5.8 to 27.2)</td>
<td>20.0 (9.0 to 35.7)</td>
<td>20.0 (8.7 to 31.0)</td>
</tr>
<tr>
<td>CD4</td>
<td>2.4 (1.2 to 5.3)</td>
<td>2.8 (1.2 to 12.0)</td>
<td>4.2 (1.8 to 18.3)</td>
</tr>
<tr>
<td>CD8</td>
<td>7.5 (2.7 to 9.8)</td>
<td>8.5 (2.3 to 16.0)</td>
<td>7.6 (2.8 to 16.4)</td>
</tr>
<tr>
<td>CD20</td>
<td>0.5 (0.0 to 1.5)</td>
<td>0.2 (0.0 to 1.8)</td>
<td>0.0 (0.0 to 1.7)</td>
</tr>
<tr>
<td>CD56</td>
<td>27.2 (12.0 to 37.3)</td>
<td>23.8 (14.1 to 41.7)</td>
<td>19.7 (11.4 to 46.0)</td>
</tr>
<tr>
<td>CD57</td>
<td>0.0 (0.0 to 0.8)</td>
<td>0.0 (0.0 to 0.3)</td>
<td>0.0 (0.0 to 0.0)</td>
</tr>
<tr>
<td>CD68</td>
<td>12.6 (6.7 to 28.0)</td>
<td>13.5 (7.0 to 25.8)</td>
<td>16.5 (8.7 to 21.0)</td>
</tr>
<tr>
<td>CD69A</td>
<td>0.0 (0.0 to 1.0)</td>
<td>0.2 (0.0 to 2.0)</td>
<td>0.2 (0.0 to 1.7)</td>
</tr>
<tr>
<td>CD45RA</td>
<td>0.0 (0.0 to 0.3)</td>
<td>0.0 (0.0 to 0.7)</td>
<td>0.0 (0.0 to 0.0)</td>
</tr>
<tr>
<td>CD45RO</td>
<td>3.9 (2.5 to 8.5)</td>
<td>13.3 (5.8 to 30.0)</td>
<td>13.3 (4.0 to 39.0)</td>
</tr>
</tbody>
</table>

The median and range of the number of positive cells per ×400 field of each leucocyte population are shown.
p = 0.012; percentage, NP 27.6 (5.6)%, CHM 45.6 (3.1)%, p = 0.0058) (figs 1, 2, and 3; tables 2 and 3). The percentage of CD3+ T cells in complete hydatidiform moles was also increased significantly compared with partial hydatidiform moles, at 36.6 (3.0)%, p = 0.0355. Similarly, the number and percentage of CD4+ T cells were significantly increased in complete hydatidiform moles compared with normal decidua: number, NP 2.7 (0.5), CHM 6.5 (1.3), p = 0.0058; percentage, NP 5.6 (1.1)%, CHM 13.8 (2.1)%, p = 0.0032 (figs 1, 2, and 4; tables 2 and 3). As in normal pregnancy CD45RA+ cells were virtually absent from partial and complete molar pregnancy decidua.

The numbers and percentage of CD8+ T cells also increased in partial hydatidiform moles compared with normal early pregnancy decidua, the differences were not significant. The ratio of CD8+ to CD4+ T cells was significantly increased in complete hydatidiform mole (1.6:1) compared with normal early pregnancy decidua (3.2:1, p = 0.02) and partial mole (2.4:1, p = 0.0378).

The numbers and percentages of CD45RO+ cells were significantly increased in both partial (number, 15.1 (2.2), p = 0.0001; percentage, 27.7 (2.7)%, p = 0.0002) and complete (number, 17.4 (2.9), p = 0.0004; percentage, 34.3 (4.7)%, p = 0.0002) hydatidiform mole compared with normal first trimester decidua (number 4.2 (0.6), percentage 8.8 (1.3)%). As in normal pregnancy CD45RA+ cells were virtually absent from partial and complete molar pregnancy decidua.

In contrast, there were no significant differences in the number or percentage of CD8+ T cells between complete and partial molar and normal pregnancy decidua (figs 1, 2, and 5; tables 2 and 3). Although CD68+ macrophages increased in complete hydatidiform moles compared with normal early pregnancy decidua and partial hydatidiform mole, this difference was not significant. As in normal decidua, CD79a+ and CD20+ B cells and CD57+ natural killer cells were rare in molar pregnancy decidua (figs 1 and 2; tables 2 and 3).

DOUBLE IMMUNOHISTOCHEMICAL LABELLING

Double immunohistochemical labelling confirmed the increase in the numbers of CD3+ cells in complete hydatidiform moles compared with normal decidua. The numbers and percentages of CD3+, CD4+ and CD8+ T cells also increased in partial hydatidiform moles compared with normal early pregnancy decidua, the differences were not significant. The ratio of CD8+ to CD4+ T cells was significantly decreased in complete hydatidiform mole (1.6:1) compared with normal early pregnancy decidua (3.2:1, p = 0.02) and partial mole (2.4:1, p = 0.0378).

The numbers and percentages of CD45RO+ cells were significantly increased in both partial (number, 15.1 (2.2), p = 0.0001; percentage, 27.7 (2.7)%, p = 0.0002) and complete (number, 17.4 (2.9), p = 0.0004; percentage, 34.3 (4.7)%, p = 0.0002) hydatidiform mole compared with normal first trimester decidua (number 4.2 (0.6), percentage 8.8 (1.3)%). As in normal pregnancy CD45RA+ cells were virtually absent from partial and complete molar pregnancy decidua.

The numbers and percentage of CD56+ cells (eGLs) in decidua from complete hydatidiform moles did not differ significantly from those in partial moles and normal first trimester decidua. Although CD68+ macrophages increased in complete hydatidiform moles compared with normal early pregnancy decidua and partial hydatidiform mole, this difference was not significant. As in normal decidua, CD79a+ and CD20+ B cells and CD57+ natural killer cells were rare in molar pregnancy decidua (figs 1 and 2; tables 2 and 3).
of CD3 cells that coexpressed CD45RO increased in both partial and complete hydatidiform moles (table 4); up to 50% of CD3 cells in molar pregnancy decidua coexpressed CD45RO, compared with 30% in normal early pregnancy decidua. In contrast, the number of decidual CD56+ cells that coexpressed CD45RO was comparable in normal pregnancy and in partial and complete hydatidiform moles (data not shown).

Discussion

Our analysis of decidual leucocytes in normal first trimester pregnancy is in agreement with previous studies of frozen tissues. T cells accounted for a lower proportion of the stromal leucocytes compared with the CD56+ granulated lymphocytes but, as reported previously, many decidual T cells were CD45RO+ memory cells. Decidual T cells have also been reported to express various activation markers including HLA-DR, CD69, and interleukin (IL)-2 receptor α and β, although expression of the α IL-2 receptor subunit has been disputed. Despite this evidence of activation, to date there have been no functional studies of decidual T cells in normal pregnancy. It has been proposed that Th2-type cytokines are necessary for successful pregnancy, whereas Th1 type cytokines may be deleterious. Although it has been reported that a wide range of cytokines has been produced in human uteroplacental tissues, the cytokine profile of decidual T cells has not been fully defined. However, recent studies of T cell clones from early pregnancy decidua have indicated defective production of Th2 type cytokines in unexplained recurrent miscarriage compared with normal first trimester pregnancy. It can be hypothesised that the abnormal trophoblast proliferation and invasion in gestational trophoblastic neoplasia may be associated with an abnormal decidual leucocyte profile and hence an altered local cytokine milieu.

As complete hydatidiform mole is androgenetic, it appears likely that molar trophoblast may stimulate an altered maternal immune response. Our study extends previous immunohistochemical studies of T cell populations in complete molar pregnancy decidua. CD3+ T cells accounted for a larger percentage of the leucocytes, with a dramatic increase in the number and proportion of CD45RO+ cells. Moreover, the CD4+ T cell population which accounts for only a small proportion of decidual T cells in normal first trimester decidua was increased significantly in complete molar pregnancy, with an altered CD8:CD4 ratio of 1.6:1 in complete moles compared with 3.2:1 in normal pregnancy. These findings conflict with a previous study which reported that, although CD4+ T cells increased in complete molar pregnancy decidua, CD8+ T cells formed only a minority decidual T cell population, comprising only one quarter to one third of the total number of T cells. This may reflect the use of different techniques: the latter

<table>
<thead>
<tr>
<th>Samples</th>
<th>Single labelled CD3+ cells/field</th>
<th>Double labelled CD3+CD45RO+ cells/field</th>
<th>Total number of CD3+ cells/field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal decidua</td>
<td>5.80 (0.80)</td>
<td>2.33 (0.33)</td>
<td>8.17 (1.17)</td>
</tr>
<tr>
<td>Partial hydatidiform mole</td>
<td>10.13 (0.63)</td>
<td>8.25 (0.50)</td>
<td>18.13 (8.8)</td>
</tr>
<tr>
<td>Complete hydatidiform mole</td>
<td>11.09 (1.92)</td>
<td>10.42 (1.25)</td>
<td>21.50 (3.17)</td>
</tr>
</tbody>
</table>

Values are mean (SEM) number of cells per ×400 field (three to six fields were counted for each case).
study used frozen tissues and an indirect immunoperoxidase method, whereas the present study used a large number of paraffin embedded tissues with heat mediated antigen retrieval and a more sensitive avidin-biotin peroxidase technique. Moreover, CD4+ macrophages may have been included in the assessment of CD4+ T cells. Our findings of altered activated CD45RO+ T cells in molar pregnancy decidua suggest that an altered maternal immune response may be induced by molar trophoblast. The mechanisms of T cell activation in normal pregnancy decidua are unknown. Extravillous trophoblast in normal pregnancy expresses a non-classical I MHC antigen, HLA-G, which shows limited polymorphism. HMC antigen expression by molar trophoblast has been less extensively investigated but evidence indicates similar MHC antigen expression by molar trophoblast. Moreover, expression of HLA-G by choriocarcinoma cell lines has been well documented. It is possible therefore that the invasive trophoblast within decidua leads to activation of decidual T cells. Despite the similarities in MHC expression, the present results suggest that this response may differ for molar trophoblast.

The function of T cells in normal pregnancy decidua is unknown, although it is reasonable to suggest that they contribute to local cytokine production. It is not known whether the increased numbers of CD4+ and CD45RO+ T cells reflect altered cytokine production which could enhance either cell-mediated cytotoxicity or humoral immunity in response to molar trophoblast. It has been reported that cytotoxic trophoblast cells in normal pregnancy and complete hydatidiform mole express Fas ligand (FasL) which may induce apoptosis of decidual activated CD95 (Fas) + T cells. The presence of increased numbers of activated T cells in molar pregnancy decidua suggest that there may be an increased turnover rate of these activated T cells in response to the molar trophoblast. The absence of B cells, immunoglobulin, and complement deposition at the molar implantation site suggests that humoral immunity is not the primary immune response mechanism. It appears unlikely that the alterations in decidual leucocyte populations noted in the present study reflect decidual inflammation. B cells were not a feature of molar pregnancy decidua and, moreover, none of the samples showed evidence of decidual necrosis, acute inflammatory cells, or plasma cells.

Further studies are required to clarify the role of decidual leucocytes, particularly T cells, in molar pregnancy. We are currently performing an immunohistochemical study of decidual T cell cytokine expression in molar pregnancy with the aim of elucidating the in vivo role of these cells.

We wish to thank Dr Somchaya Neungton (department of obstetrics and gynaecology, Siriraj Hospital, Bangkok, Thailand) for his kind cooperation in retrieving and providing us with hydatidiform mole tissues. We also thank Ms Helen Bell and Ms Barbara Irenes for their technical assistance.


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32 Starkey PM. Expression on cells of early human pregnancy decidua, of the p75, IL-2 and p145, IL-4 receptor proteins. *Immunology* 1991;78:64–70.


