Increased expression of Interleukin-1 beta is associated with persistence of the disease and invasion in complete hydatidiform moles (CHM)

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Summary

Complete hydatidiform moles (CHM), a post-conceptual pathologic condition of the placenta, have a high prevalence rate (12/1,000 deliveries) in Kerala, India. This study addresses the expression of IL-1 alpha and beta by immunohistochemistry in relation to persistence and invasion of the disease. Mild to moderate expression of IL-1 alpha in the villous cytotrophoblasts, syncytiotrophoblasts and decidua of the first trimester in the normal placenta and all gestational ages in the molar placenta were observed. IL-1 beta expression was observed in the extravillous trophoblasts, syncytiotrophoblasts and decidua in both the normal and molar placentae and also in the villous cytotrophoblasts and the stromal Haufbaur cells in molar placentae. Strong expression of IL-1 beta in the placenta suggests its involvement in placental physiology supporting earlier reports. Higher expression of IL-1 beta correlated well with the invasive and persistent nature of the tumour and holds potential as a marker of persistence and invasion in CHM.

Key words: Complete hydatidiform moles; IL-1α, IL-1β, Placenta; Invasion; Persistence.

Introduction

Complete hydatidiform moles (CHM) are post-conceptual pathologies of the placenta. The prevalence rate is much higher in Kerala, India when compared to other parts of the world [1]. Most often this occurs in females of reproductive age in their first or second pregnancies. The WHO and FIGO systems of classifications have identified a number of factors such as clinical presentation, cytogenetic characteristics and type of disease reported to be associated with the aggressiveness of the disease. These markers have however not taken into account the biological characteristics of the tumour. Persisting trophoblastic disease (PTD) includes the group of patients (approximately 20% [2]) whose serum beta HCG levels do not follow the normal pattern of regression after evacuation of a mole and are thought to have a high risk of malignant transformation. The host factors do not seem to be the deciding factor of persistence as recurring CHMs occur only in a very small proportion of the patients [3] and hence the biologic characteristics of the tumour might play a role here.

Early pregnancy is characterised by rapid invasion of the trophoblasts into the endometrium, growth of trophoblastic villi and increased placental size [4] . The process of invasion of trophoblasts is akin to invasion by cancer cells. In common with invasion, there is also a release of active cytokines in and around the implantation site. At times, in hydatidiform moles, the increased trophoblast invasion of the maternal tissues occurs to the extent that systemic chemotherapy is needed for regression of the tumour. The overall situation in these conditions assumes the nature of an invasive tumour.

Cytokines have been recognised as playing important roles in pregnancy [4-6]. Both Interleukins α and β are involved in the differentiation of cytotrophoblasts to syncytiotrophoblasts [7] with peak expression in the first trimester. The biological activities of IL-1 beta have been studied extensively. The major functions of this protein include inhibition of endothelial growth (8), regulation of trophoblast invasion [9, 10], regulation of prostaglandins [11], regulation of HCG secretion [12], proliferation and differentiation of trophoblasts [7, 13], control of decidualisation of endometrial cells [14], process of labour [15], regulation of metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) [10, 16]. IL1-alpha, in contrast, appears to be more involved in pro-inflammatory and immune functions [17, 18] related to immune responses to infections. Many reports show IL-1 to be produced by murine and human placenta but the cell type that is responsible for production of this protein is not yet clear. Since IL-1 beta is reported to have a role in the regulation of proteins related to invasion of trophoblasts in normal pregnancy [9, 10], we have, in this study, evaluated the expression of IL-1 alpha and beta and studied its relation to the persistence and invasion of trophoblasts in CHM.

Materials and Methods

Molar placentae from 108 patients with complete molar pregnancy and four cases with choriocarcinoma who attended the trophoblastic clinic of SAT Hospital from 1994-1996 and 112 normal placentae from terminated normal pregnancies and normal term deliveries were collected and used for this study.

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The clinical details of the patients and normal controls with regard to age, parity, gestational age, obstetric history, presenting symptoms, serum beta human chorionic gonadotrophin levels at every follow-up, and regression patterns were collected and used for analysis. Tissue was collected at the time of suction evacuation, the first line of treatment at this centre, and a part of it was fixed in 10% buffered formalin and processed for paraffin embedding and sectioning. The patients were classified into spontaneously regressing and persisting disease groups. Patients attaining normal serum βHCG levels (<10 mIU/ml) by 16 weeks and for a consecutive 2-3 weeks following evacuation were included in the spontaneously regressing disease group (Spont. Regr.). The persisting disease (PTD) group of patients comprised of [1] those who were administered chemotherapy (CT) prior to 16 weeks of evacuation due to clinical symptoms of myometrial invasion such as ultrasound findings, histology, persistingly high levels of serum beta HCG for three consecutive weeks after evacuation, and [2] those patients whose serum βHCG levels did not touch the normal levels (<10 mIU βHCG/ml) by 16 weeks and not administered chemotherapy prior to 16 weeks (slow regressing group - SLR) (Figure 1). This hospital considers a patient with serum βHCG levels of <10 mIU/ml at three consecutive evaluations between 12-16 weeks of suction evacuation as having attained normal levels and hence we have used this as a cut-off. The CT group was analysed separately as they were administered chemotherapy between 2-16 weeks of evacuation and estimation of serum βHCG at various periods of evacuation could not be used as an indicator. The invasive group of tumours included those with histological evidence of myometrial invasion.

Immunohistochemical staining

The avidin biotin immunoperoxidase assay was performed on 5 μ paraffin sections. The primary monoclonal antibodies to IL1-α and IL1-β were purchased from Oncogene Sciences, USA and the conjugated antibodies and substrate amino ethyl carbazole (AEC) were purchased from Sigma, USA. Sections to which normal mouse serum at a dilution of 1:20 was added instead of the primary antibody served as controls. The staining was developed using AEC as the chromogen and graded as: - negative (Score 1); + mild positive (Score 2); ++ moderate positive (Score 3) and +++ intense positive (Score 4) based on the intensity of >80% cells in each villi . Localization of the staining was also noted. The score for cytotrophoblasts and syncytiotrophoblasts was used to analyze the behaviour of the lesion with respect to regression. The slides were evaluated blindfolded by a second investigator (M.J) and the average of the scores was considered for analysis.

Statistical Analysis

The values are expressed as mean with standard error. Analysis of the staining intensities (score) was done using the Chi-square test and the non-parametric Mann-Whitney U Wilcoxon-Rank sum W test, p-values <0.05 were considered significant.

Results

Clinicopathological data of patients

The placentae, both normal and pathological belonged to gestational ages between 6 weeks and 36 weeks with the mean gestational age of normal placenta being 17.94±10.34 weeks and that of the pathological placenta being 15.96±6.31 weeks with 77 normal placentae and 65 molar placentae belonging to early gestation (6-12 weeks),

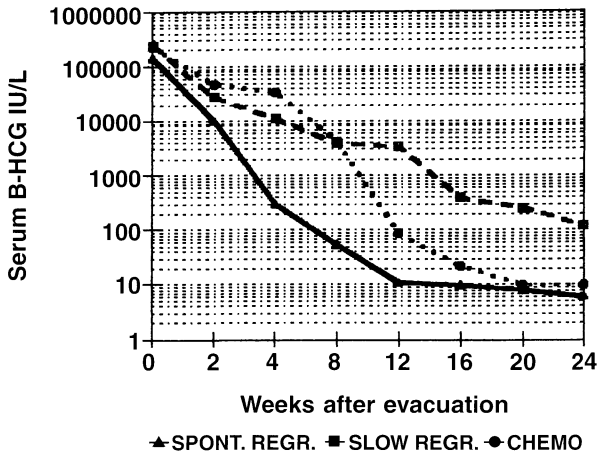

Figure 1. — Beta HCG regression curve.

15 normal placentae and 31 molar placentae belonging to mid-gestation (13 to 24 weeks), and 20 normal and 12 molar placentae belonging to late gestation (>24 weeks). Medical termination of pregnancy (MTP) in a normal condition beyond 12 weeks of gestation and molar pregnancies continuing up to the third trimester are very rare and hence the mid-gestation normal placentae and late gestation molar placentae form smaller groups. Among the molar placentae, 71 (66%) belonged to the spontaneously regressing disease group and 37 (34%) to the persisting disease group. The persisting disease group included 23 patients who were administered chemotherapy for regression of the tumour and fell in the chemotherapy group and 14 patients who were not administered chemotherapy up to 16 weeks but had either fluctuating beta HCG levels or values above normal limits (slow regressing group). Ten of the patients in the slowly regressing disease group attained normal levels of beta HCG by 30 weeks of follow-up without any chemotherapeutic intervention. The other four patients had to be administered chemotherapy at 20-24 weeks of follow-up due to increased beta HCG levels.

Immunohistochemical localization

IL1 - Alpha (IL1-α)

Mild to moderate expression of IL-1α was observed in villous cytotrophoblasts, syncytial trophoblasts and extravillous cytotrophoblasts in both normal placentae and CHM (Plate 1, Figures A-F). In those cases requiring chemotherapy and the cases of invasive lesions, the innermost layer of cytotrophoblasts were negative for IL-1α expression. The highest expression of IL-1α in normal placenta was in the first trimester with lower intensities in the second and third trimesters (Table 1). In CHM, the mean score was not very different in the three gestational age groups, and was higher than that in the normal placenta in the second and third trimesters, even though not statistically significant. In choriocarcinoma cases both the cytotrophoblasts and the syncytial trophoblasts showed mild to moderate positivity. About 40% of the decidual cells in the first trimester and 20-30% in the second and third trimester were seen to be positive for IL1-α.

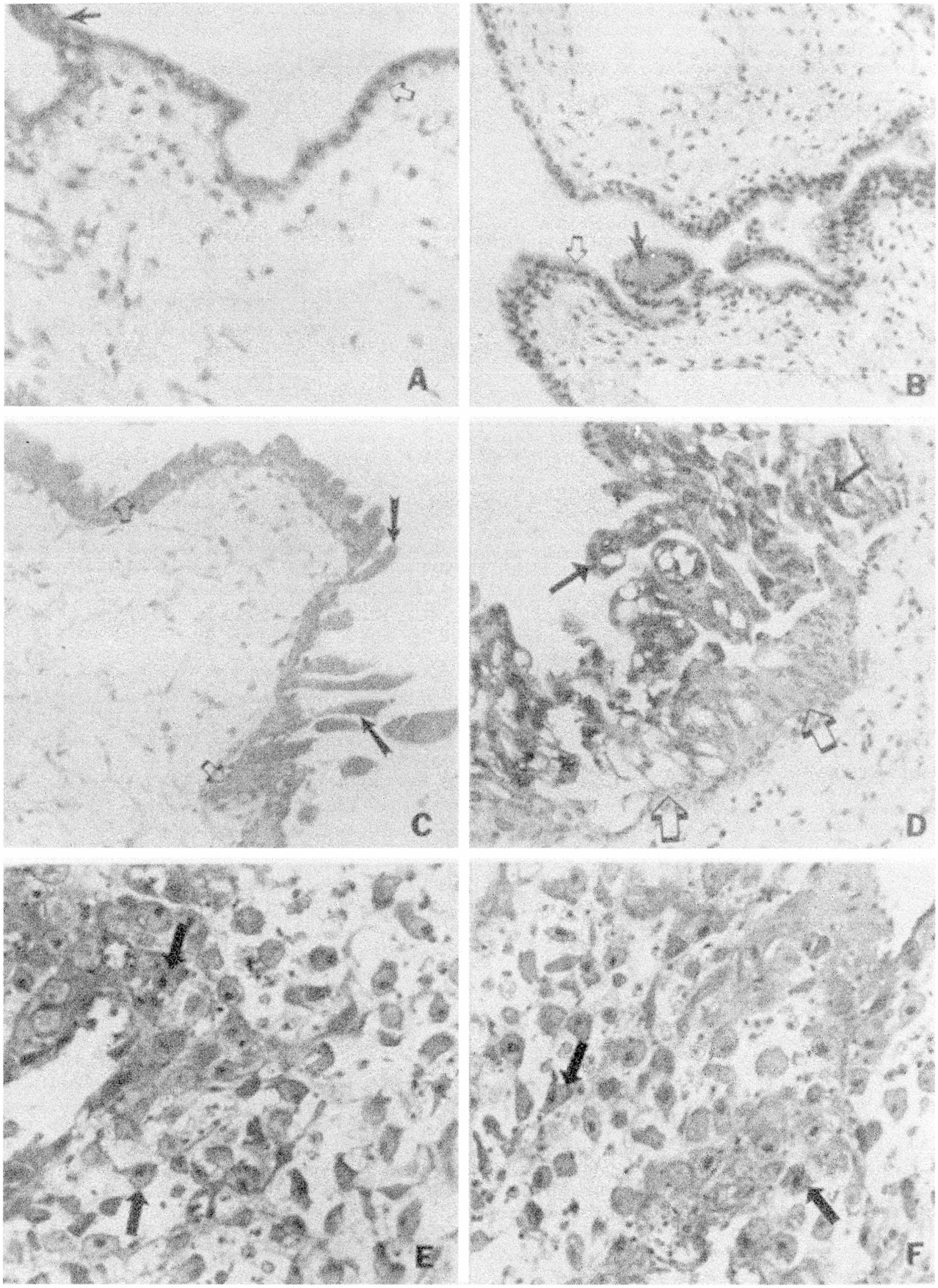

Plate 1. — Figures A, B, C, D, E, F.

For classification on the basis of regression pattern, tumours in the persisting disease group showed a trend toward higher expression (4.13±0.38 Vs 3.59±0.24 in the spontaneously regressing disease group), even though not statistically significant (p value 0.10). The comparatively flat line seen in Figure 2 demonstrates this. No statistically significant difference (p>0.50) was noticed in the expression of IL-1 alpha in the invasive group of tumours (Table 3), slow regressing tumours or those tumours belonging to the slow regressing disease group which showed spontaneous regression.

Interleukin 1- Beta (IL-1β)

The IL1-β expression was comparatively stronger both in normal placentae and CHM (Table 4) in comparison to IL-1 alpha. The protein was localised in the extravillous cyto- and syncytiotrophoblasts and decidua in both normal and CHM (Plate 2, Figures A-F). In CHM, however, the expression was also noticed in the villous cytotrophoblasts and the stromal cells. The innermost layer of cytotrophoblasts were negative for IL-1 beta expression in all the cases requiring chemotherapy and invasive lesions. The staining was mostly diffuse, cytoplasmic and mainly granular. The expression of IL1-β by the cytotrophoblasts in choriocarcinoma was moderate to intense in all the samples.

Correlation of anti IL-1β staining with prognosis of these tumours showed this to be a marker of persistence of the disease. Tumours belonging to the persisting disease group showed significantly higher expression of this protein (Table 2 & Figure 2 - Corr. Coeff. 0.4787, p value 0.0001) and it was associated with longer time for attaining normal serum beta HCG levels (Figure 3, p value 0.014). The mean staining score in the persisting disease group was 5.75±0.28 in comparison to 4.06±0.21 in the spontaneously regressing tumours. The over-expression in the persisting disease group was highly significant when compared to the spontaneously regressing disease group (p value 0.001). All cases that needed chemotherapy for regression showed moderate to intense expression while those that regressed without chemotherapeutic intervention including those with a longer follow -up time (24-30 months) showed only mild to moderate expression. The tissues of the four cases in the slow regressing disease group that had to be administered chemotherapy at a later stage had higher expression of IL-1 beta; 89% of the tumours in the group had to be administered CT, based on clinicopathological findings while 54% of the slow regressing disease group and only 4% of the spontaneously regressing tumours had moderate to high expression of IL-1β . Expression of IL1-β showed a significant correlation with the invasive potential of the tumour (Table 3 & Figure 4) with a correlation coefficient of 0.2735 and p value of 0.036. The relative risk of tumours expressing a high amount of IL-1 beta to be invasive was 5.40.

Table 1. — *IL-1 Alpha expression - Normal placenta vs CHM.*

Gest. age (wks)	Placenta		CHM		p value
	No. of cases	Mean ± SE	No. of cases	Mean ± SE	
6-36	112	3.68±0.20	108	3.83±0.18	0.57
<13	77	4.06±0.22	65	3.89±0.29	0.64
13-24	15	2.33±0.33	31	3.78±0.24	0.06
>24	20	2.66±0.42	12	4.0±0.71	0.12

Table 2. — *IL-1 Expression vs prognosis in CHM.*

Type of regression	No. of cases	IL-1Alpha	IL-1 Beta
I. Spontaneous Regression	71	3.59±0.24	4.06±0.21
II. Persisting Disease	37	4.13±0.38	5.75±0.28
(a) Slow Regression	14	4.11±0.38	5.50±0.30
(b) Chemo Group	23	4.18±0.57	6.36±0.59
III Choriocarcinoma	4	4.16±0.52	6.83±0.61

P value	IL-1 Alpha	IL-1 Beta
Spontaneous regression vs persisting disease	0.10	0.001
Spontaneous regression vs slow regression	0.22	0.0001
Spontaneous regression vs chemotherapy	0.27	0.003
Slow regression vs chemotherapy	0.88	0.09
Spontaneous regression vs choriocarcinoma	0.05	0.001

Table 3. — *Interleukin expression in invasive and non-invasive CHM.*

Type	No.	IL-1α	IL-1β
Invasive	19	3.72±0.21	4.69±0.188
Non-invasive	89	3.85±0.65	5.90±0.51
p-value (χ^2 test)		0.50	0.022

Table 4. — *IL-1 Beta expression - Normal placenta vs CHM.*

Gest. age (wks)	Placenta		CHM		p value
	No. of cases	Mean ± SE	No. of cases	Mean ± SE	
6-36	112	4.79±0.21	108	4.77±0.18	0.93
<13	77	4.95±0.20	65	4.76±0.30	0.71
13-24	15	4.33±0.95	31	4.85±0.24	0.48
>24	20	4.00±1.03	12	4.00±0.63	0.77

Plate 1. Immunohistochemical staining of normal and molar placenta with antibody to IL-1α

A. Normal placental villi showing moderate staining of cytotrophoblasts and syncytial trophoblasts (arrows) X 250.

B. Molar placenta section from spontaneously regressing tumours showing mild staining of cytotrophoblasts and moderate staining of syncytiotrophoblasts (arrows) X 250.

C. Slow Regressing molar placenta with negative staining of inner cytotrophoblasts and mild staining of syncytiotrophoblasts (arrows) X 250.

D. Section from molar placenta requiring chemotherapy for regression showing negative inner cytotrophoblasts and moderately positive syncytiotrophoblasts (arrows) X 250.

E. Decidual tissue from normal pregnancy showing mild positivity to IL-1α X 250.

F. Decidual tissue from molar pregnancy showing moderate diffuse cytoplasmic localisation of IL-1α X 250.

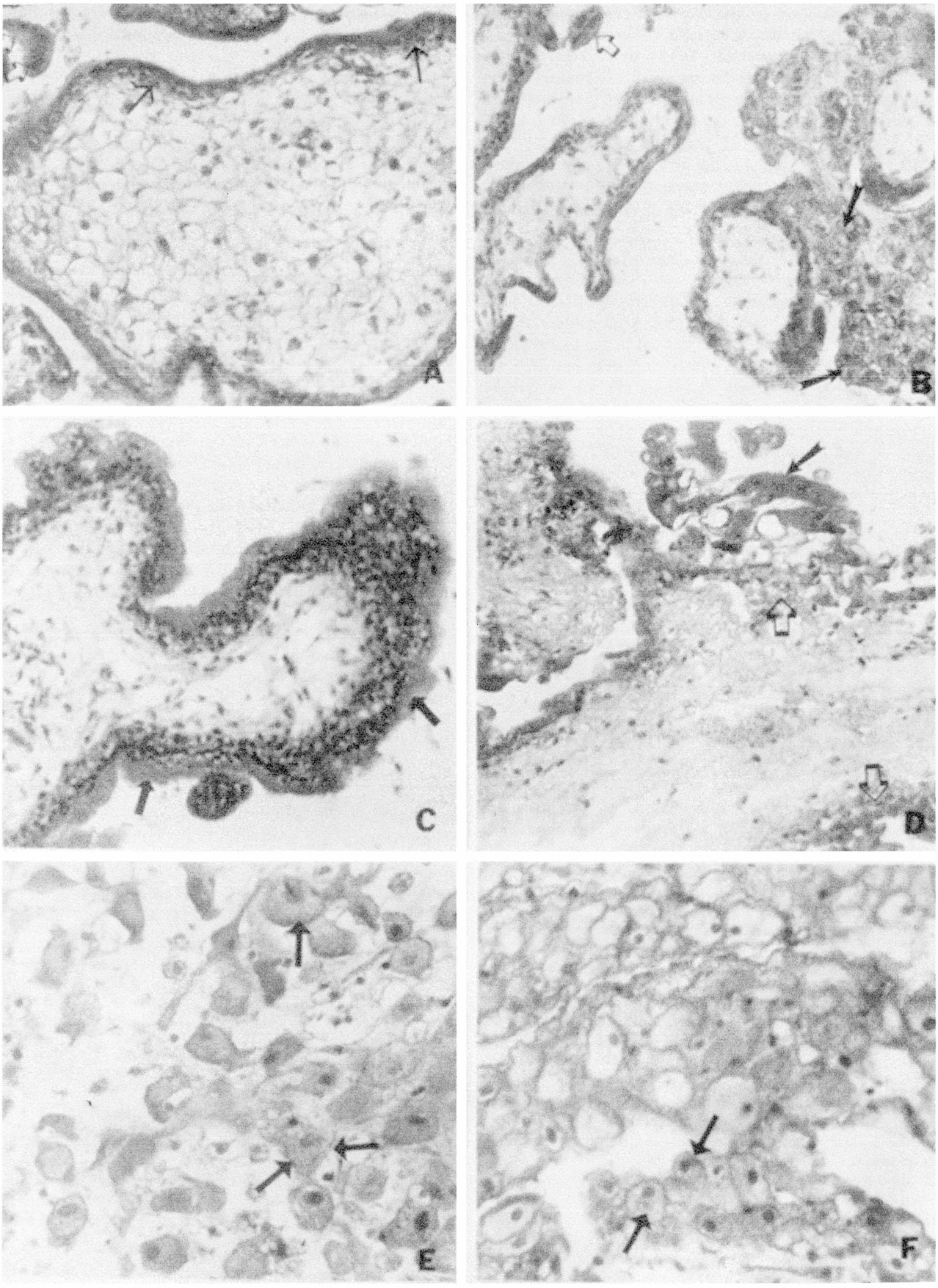

Plate 2. — Figures A, B, C, D, E, F.

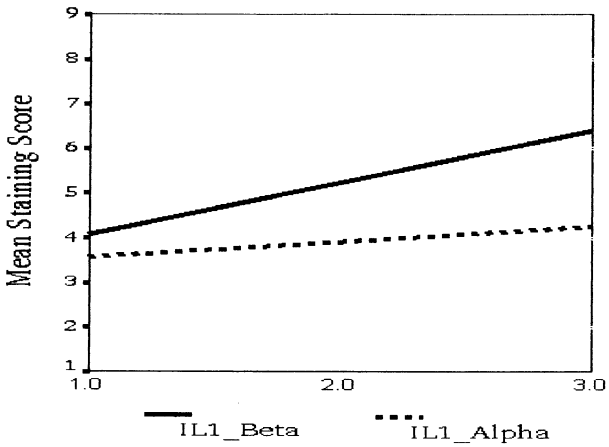

Figure 2. — Interleukin 1 β and α in complete hydatidiform moles - correlation with prognosis. 1) Spontaneous regression. 2) Slow regression. 3) Chemotherapy.

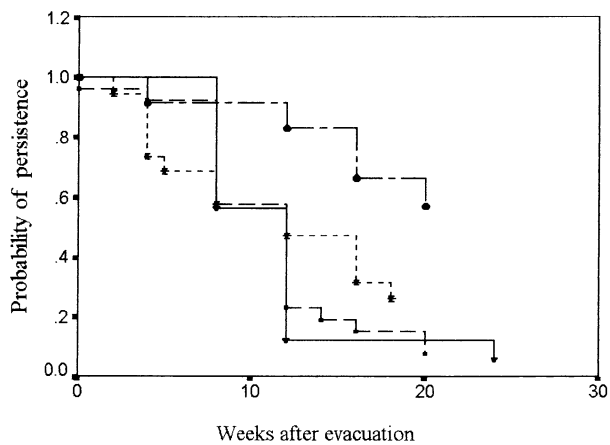

Figure 3. — Interleukin 1 β expression in relation to persistence of disease •----• intense expression, _----_ moderate expression, □----□ mild expression, ⩛----⩛ negative expression.

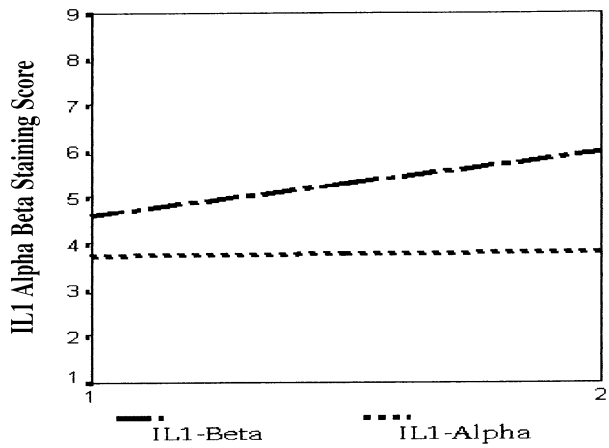

Figure 4. — Interleukin 1 β and α expression in relation to invasion in CHM 1) Non-Invasive. 2) Invasive.

Discussion

Placenta is a major source of interleukin, a multifunctional cytokine that mediates immune responses and other physiological functions in the body. The maternal tissue, the decidua, also produces high amounts of IL1. Our observations support some of these early findings and we have demonstrated the presence of IL1 alpha and beta in normal placenta in all the gestational periods. The status of IL1α and β in pathological placenta like in hydatidiform moles is not available in the literature; our study shows that the molar tissue secretes IL1α and β in higher concentrations when compared to normal placenta. IL1α and β are reported to have a strong role in labour independent of bacterial infection [19] even though we have not noticed a higher expression of this protein in term placentae when compared to placenta belonging to earlier gestational periods. IL1β is also attributed to various other functions including regulation of trophoblast invasion [9, 10, 16], proliferation and differentiation of trophoblasts [7, 13], regulation of proteolytic enzymes of the metallo- and serine proteases family [10, 16, 20], immunoregulatory role in the human endometrium [21], regulation of prostaglandins, beta HCG production, etc. It has also been reported that IL-1β could inhibit progesterone-induced decidualization of human endometrial stromal cells in vitro, as assessed by morphological changes and prolactin production [17, 18, 23, 24]. Interleukin-1 released from trophoblast cells may alter cellular growth, differentiation and function in an autocrine or paracrine manner [25]. All these reports point towards the importance of IL1α and β in trophoblast functioning and invasion and hence could be of importance in the maintenance of the proliferative and invasive capacity of trophoblasts in CHM.

The present results show IL1-α to be expressed in both normal and molar placenta of all gestational ages. The degree of expression was mild to moderate in the first trimester in normal placenta and negative in the second and third trimesters. In contrast to this, a mild to moderate expression of this protein was seen throughout gestation in the molar placenta. The increase was however not statisti-

Plate 2. Immunolocalisation of IL-1β in normal and molar placenta and decidua

A. Normal placenta (first trimester) showing moderate cytoplasmic staining of cytotrophoblasts and syncytiotrophoblasts (arrows) X 250.

B. Molar placenta (spontaneously regressing) showing moderate localisation of IL-1 beta in the cytotrophoblasts and syncytiotrophoblasts.

C. Molar placenta (slow regression) showing intense diffuse staining of cytotrophoblasts and syncytiotrophoblasts X 250.

D. Molar placenta requiring chemotherapy for regression showing negative inner cytotrophoblasts and intensely stained outer cytotrophoblasts and syncytiotrophoblasts X 250.

E. Decidual tissue from normal pregnancy showing moderate expression of IL-1 beta X 400.

F. Decidual tissue from molar pregnancy showing moderate granular positivity for IL-1β X 400.

cally significant. The expression showed no correlation to the type of the mole or to the regression pattern of the tumour suggesting that IL1-α is probably not directly related to trophoblast functioning. IL1-α appears to be more related to infection than trophoblast function as reported by Yamaguchi *et al.* [25]. Romero *et al.* [26] reported that term decidua in vivo can produce IL-1α and to a lesser extent, IL-1β in response to bacterial endotoxin.

In this study IL1-β was found to be present in the cytotrophoblasts, syncytiotrophoblasts and decidual cells both in normal placenta and molar placenta and it is probably responsible for autocrine stimulation of the trophoblasts. The Haufbaur cells of the stroma and villous cytotrophoblasts were found to express this protein only in the pathological condition. The finding is in agreement with those of others [21, 22, 26, 27] in conditions other than hydatidiform moles. The status in CHM has not been reported earlier. Placental cells produce IL-1β type bioactivity [23] and immunoreactivity although the mRNA expression is minimal [22]. In our study, IL-1β expression was noted in first, second and third trimesters in both normal placenta and hydatidiform moles. A higher expression of IL-1β in villous trophoblasts, extravillous trophoblasts and in the decidua was noted in trophoblastic tumours especially in the invasive and persisting tumours. There is strong evidence that this cytokine is therefore present in the placental environment and with its reported association with proliferation, differentiation and invasion of trophoblasts, these results suggest that its expression could be a potential marker of invasion and persistence of the disease.

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