COMMENT

Response to an article by Hamilton *et al.* on 'Effects of colony stimulating factor-1 on human extravillous trophoblast growth and invasion'

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We wish to comment on the article by Hamilton *et al.* 1998.

Table 1 shows the similarities and differences between the observations made in the two papers (Lewis *et al.* 1996, Hamilton *et al.* 1998) which have investigated the roles of colony-stimulating factor-1 (CSF-1) and its receptor (c-fms) in human extravillous cytotrophoblast (EVCT). Both types of EVCT show the components of a CSF-1/c-fms autocrine loop, and it is clear that there is no role for CSF-1 in controlling EVCT invasion (Table 1). However, in primary cells (designated EVT), CSF-1 was a positive regulator of cell proliferation (Hamilton *et al.* 1998), whereas in immortalised TCL-1 cells (and in BeWo choriocarcinoma) CSF-1 appeared to be an inhibitor of such proliferation (Lewis *et al.* 1996).

There are a number of factors which may contribute to this difference in the data obtained, but we must firstly point out that Hamilton *et al.* have not reported our previous data accurately. In the last paragraph of the discussion they state "... those cells (TCL-1 cells) also exhibited an autocrine CSF-1 loop which enhanced proliferation." This is the opposite of what we reported (Lewis *et al.* 1996), that "Proliferation of TCL-1 cells .... was elevated in response to treatment with a CSF-1 neutralising antibody." (abstract). We therefore suggested that CSF-1 may be a differentiation or maturation factor which suppresses the proliferation of trophoblasts (Aplin 1991).

Possible explanations for the differences in effects of CSF-1 include the following:

(1) EVT were obtained by culture of adherent cells and explants from minced first trimester villi (Hamilton *et al.* 1998), whereas TCL-1 were from chorionic membranes and immortalised with the SV40 large-T antigen (Lewis *et al.* 1996). There may be a change in trophoblast response to CSF-1 depending on gestational age, intrauterine location or, as described by Hamilton *et al.*, the fact that TCL-1 were immortalised.

(2) TCL-1 cells did not respond to CSF-1, and the levels of CSF-1 released were sufficient to saturate the c-fms receptor (Lewis *et al.* 1996). The release of CSF-1 from EVT cells was not quantified, but the response of the cells to CSF-1 *in vitro* suggested that the receptor would not be saturated. The quantity of CSF-1 needed (62·5 ng/ml, or ~750 nM) is higher than the accepted affinity of the c-fms receptor (~30 nM).

(3) The proliferation indices of the cells seem to differ markedly. In serum-free medium, EVT incorporated 400–1000 c.p.m./6 h (Hamilton *et al.* 1998). TCL-1 cells incorporated about 300 000 dpm/h (our unpublished data), a rate substantially greater. The EVT cells were examined on day 5 of culture, in contrast to TCL-1 cells

Table 1 Comparison between results from Hamilton *et al.* 1998 and Lewis *et al.* 1996

	Hamilton *et al.* 1998	**Lewis *et al.* 1996**	**Agreement**
Cell type	Extravillous trophoblast	Extravillous trophoblast	Yes
CSF-1	Yes (mRNA)	Yes (10–30 pM protein)	Yes*
c-fms (mRNA)	Yes	Yes	Yes
Addition of:			
CSF-1	Growth increased	No effect	No
Ab to CSF-1	Growth decreased	Growth increased	No
CSF-1, Ab to CSF-1, Ab to c-fms	*In vitro* invasion and balance between MMPs and TIMPs unaffected	MMP activity unaffected	Yes

*This may be an important point. If the EVT cells released much lower levels of CSF-1, which did not saturate the receptor, effects of exogenous CSF-1 would be expected. This still would not exlain why CSF-1 was a positive effector in Hamilton *et al.* 1998 and a negative effector in Lewis *et al.* 1996.

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(days 2–4 of culture). Our unpublished data suggest that TCL-1 proliferation decreased markedly in confluent populations, and this may contribute to the difference in proliferation obtained.

There must be a major difference in the coupling of c-fms to second messenger pathways in these two cell-types, such that in EVT the link is to transcription factors (Hamilton *et al.* 1998), and in TCL-1 cells to differentiation pathways (Lewis *et al.* 1996). The cells described in these two papers may together provide useful models for the further study of the role of the CSF-1/c-fms axis in EVCT.

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REPLY FROM AUTHOR

Possible explanation for opposite responses of EVT and TCL-1 cells to endogenous CSF-1

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We apologize for inaccurately quoting Lewis *et al.* (1996) in our recent paper by Hamilton *et al.* (1998) as CSF-1 having an autocrine growth-stimulating loop in their immortalized extravillous trophoblast TCL-1 cell line, similar to that observed by us (Hamilton *et al.* 1998) in normal extravillous trophoblast (EVT) cells.

In their study (Lewis *et al.* 1996), a growth stimulation was noted in the presence of CSF-1 neutralizing antibody indicating an anti-proliferative/differentiation-inducing role of endogenous CSF-1 for TCL-1 cells. The table included in the article above accurately compares the results of the two studies. In spite of the fact that both normal EVT cells (Hamilton *et al.* 1998) as well as TCL-1 cells (Lewis *et al.* 1996) express CSF-1 receptor and produce CSF-1 (although at different levels), the reasons for opposite responses of these cells to endogenous CSF-1 remain unclear. The most likely explanation is the genetic alterations induced by SV40 Tag immortalization in the case of TCL-1 cells. We have also derived two independently immortalized EVT cell lines, HTR-8/SVneo (Graham *et al.* 1993) and RSVT2/C (Khoo *et al.* 1998), by introducing SV40 Tag gene into a normal, mortal EVT cell line HTR-8, which was one of the cell lines used in our CSF-1 study (Hamilton *et al.* 1998). Similar to TCL-1 cells (Lewis *et al.* 1996), the proliferation rate of HTR-8/SV_{neo} cells is much higher than that of HTR-8 cells (Graham *et al.* 1993) and remains unaltered in the presence of exogenous CSF-1 (author's unpublished data). However, the possible effect of endogenous CSF-1 on proliferation of the cells remains to be tested by addition of CSF-1 neutralizing antibody or CSF-1 receptor blocking antibody. These immortalized extravillous trophoblast cell lines show a premalignant phenotype (Khoo *et al.* 1998*a*,*b*) and present as excellent tools for studies of molecular/genetic mechanisms underlying trophoblastic tumor progression.

References

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