Control of fetal survival in CBA x DBA/2 mice by lymphokine therapy

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Summary. In this study, we examined the effect of injecting various cytokines. We report here that tumour necrosis factor (TNF)α, γ-interferon and interleukin 2 (IL-2) can, in some circumstances, increase fetal resorption rates in abortion-prone (CBA/J × DBA/2) and non-abortion prone (CBA/J × BALB/c,C3H × DBA/2) matings: 1000 units TNF enhanced resorptions from 43 to 79% in CBA × DBA/2, from 7 to 89% in CBA × BALB/c, from 5 to 47% in C3H × DBA/2. The effect was both gestational age- and dose-dependent. Gamma interferon and R-IL-2 enhanced resorptions from 38 to 68% and 76% respectively in the CBA/J × DBA/2 mating combination, whereas the rates in CBA/J × BALB/c matings were enhanced from 6 to 44% and 55%. Lipopolysaccharide (LPS), which is known to lead to the release of TNF-α, had a similar effect, leading to gestational age- and dose-dependent enhancement of resorptions up to 100%.

However, cytokines of the CSF family, including IL-3 and GM-CSF, increased the chances of fetal survival when injected into abortion-prone mice, e.g. reducing resorption rates in the abortion-prone CBA/J × DBA/2 mating combination from 55 to 22% (IL-3), and 47 to 8% (GM-CSF). They also increased fetal and placental weight and, in particular, expanded the spongiotrophoblast zone in the placenta. The latter observations may be due to a direct trophic influence on placental cells, perhaps through a cytokine cascade, or an indirect effect due to inhibition of natural killer (NK)-like cells, or both. Whatever the mechanism, these results may find practical application in influencing reproductive outcome in women and other species.

Keywords: abortion; mice; lymphokines; induction; treatment

Introduction

There is increasing evidence that some recurrent abortions in women might be preventable by immunization of the mother against paternal or third party alloantigens (Taylor & Faulk, 1981; Mowbray *et al.*, 1985). Ethical concerns limit the extent of studies on this subject in women but there now exist several models of pregnancy failure in mice and its correction by lymphocyte alloimmunization.

The best studied model is the mating of CBA females with DBA/2 males: CBA/J, but not C3H-HeJ, females have an abnormal resorption rate when mated with DBA/2 males (Clark *et al.*, 1980), but when CBA/J females are mated with BALB/c males, the fetal resorption rate is normal (Chaouat *et al.*, 1983). In this system, there is suggestive evidence that abortion is mediated by asialo GM1-positive natural effector cells (Chaouat *et al.*, 1987; Baines & de Fougerolles, 1988). If

CBA/J females are immunized against BALB/c lymphocytes before mating, the high resorption rates in CBA/J mice mated with DBA/2 males are prevented (Chaouat *et al.*, 1983, 1987, 1988a, b, c) and there is no longer accumulation of asialo Gm1 + cells in the uterus (Gendron & Baines, 1988). Protection against abortion can be adoptively transferred by CBA/J anti-BALB/c immune T cells, or T cells from the spleens of CBA/J mice that were multiparous by repeated mating to BALB/c males (Chaouat *et al.*, 1985). Furthermore, T cell-derived cytokines such as IL-3, GM-CSF and CSF-1 were strongly supportive of the in-vitro proliferation of placental cells (Athanassakis *et al.*, 1987; Armstrong & Chaouat, 1989), and, conversely, tumour necrosis factor (TNF) -α and -β proved to be cytostatic for these placental cells *in vitro*. In addition, in-vivo depletion of T cells enhanced resorption rates in CBA females mated with DBA/2 males as well as in the non-abortion prone CBA × BALB/c mating (Chaouat *et al.*, 1988a). Further support for a trophic role of activated T cells *in vivo* came from the observation that depletion of T cells in the autoimmune MRL lpr/lpr strain corrected placental hypertrophy and placental phagocytic activity was then normal. This observation was corroborated by the finding that transfer of cells from MRL lpr/lpr mice into H-2k-compatible CBA/J females prevented the CBA × DBA/2 pregnancy from showing increased fetal resorption (Chaouat *et al.*, 1988a, b). Since these mice are known to produce excess IL-3, and autoantibodies with IL-3-like activity, and based on the aforementioned evidence, we decided to test directly various lymphokines, all of them products from activated T cells, for their effect on fetal resorption in CBA/J mice mated with DBA/2 males.

Materials and Methods

Mice. CBA/J mice were obtained from Iffa Credo (L'Arbresle, France) at the age of 8 weeks. They were kept for another 2 weeks in our animal facility before mating. The day of observing a vaginal plug was termed Day 0·5 of pregnancy.

Since the rate of resorption increases with ageing or environmental conditions (Chaouat *et al.*, 1987, 1988a; Hamilton & Hamilton, 1987) mice were in some cases aged a further 5 weeks before mating in a conventional room where abortions were known to be higher (Chaouat *et al.*, 1987, 1988a) than in the gnotobiotic facility. C3H/HeJ, C57BL/6, DBA/2 and BALB/c.J mice were obtained from the IRSC, Villejuif, France, at the age of 8 weeks, and also maintained for 2 weeks before use.

For experiments performed in Canada 6–8-week-old CBA/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and maintained until 12 weeks of age when they were mated.

Fetal resorptions were assessed on Day 14·5 of pregnancy, unless otherwise stated. In some experiments, animals were allowed to deliver to confirm the anti-abortive effect by direct assessment of viable offspring. This technique suffers from the fact that some pregnant mothers eat their offspring and placentae immediately after birth: the mean litter size was therefore determined on Day 1, as the 'surviving' litter size. Animals which had clearly eaten their progeny were excluded from the experiment. There was no indication that any treatment which we employed had an influence on this type of behaviour.

Lymphokines and reagents. Purified GM CSF was obtained from the P338 D1 cell line as described by Dy *et al.* (1987). Recombinant murine GM-CSF was purchased from Genzyme Inc. (Boston, MA, USA) directly or via Tebu France (Le Perray, Yvelines, France). Recombinant human GM-CSF (produced in CHO cells) was a kind gift from the Genetics Institute, Boston, MA, USA. All recombinant materials were purified to homogeneity by the manufacturers, and did not contain other lymphokines or lipopolysaccharide (LPS) that might interfere with the assay. Lipopolysaccharide (Difco, via Sigma France, itself via OSI France, Paris, France) extracted from *Salmonella enteritidis* was from a batch pretested in various LPS and LPS/TNF assays, and was a kind gift of Dr M. Parand, Institut Biomédical des Cordeliers, Paris, France. Recombinant human TNF-α, murine and human IL-2, and murine γ-interferon were kindly donated by Dr J. Wietzerbin, Institut Curie, Paris, France. Rabbit polyclonal antibody against murine GM-CSF was kindly donated by the Glaxo Institute for Molecular Biology, Geneva, Switzerland. Purified IL-3 was prepared from the WEHI cell line as described by Dy *et al.* (1987).

LPS was injected i.v. at 1 μg on Day 7 or 12, or 3 μg on Day 15. Mice were killed on Day 12 when injection was performed on Day 7, on Day 16 for injection performed on Day 12, or Day 19 for injection performed on Day 15. For TNF experiments, each group was injected i.v. with 1000 units of human recombinant TNF-α or saline on Day 7·5 of gestation, and mice were examined on Day 14·5 for fetal resorption. Alternatively, 1000, 2000 or 3000 units TNF were injected on various days of gestation and mice were checked on Day 12 (injections until Day 10·5) or 17 (injection on Day 13·5) or 19·5 (just before parturition) for injection on Day 17·5. Gamma interferon was given at 1500 units i.p. on Day 7·5 of gestation, and R-IL-2 was given at 2000 units i.p. on Days 6·5, 8·5 and 10·5 of gestation. Mice were killed on Day 14·5. Other mice received 600 units IL-3 i.p. on Day 6·5, 8·5 or 10·5 and were killed on Day 14·5.

GM-CSF was injected i.p. as (a) 400 HCSF units in 0·2 ml saline on Days 6·5, 8·5 or 10·5 or (b) 200 HCSF units in phosphate-buffered saline (PBS) on Day 6·5, 8·5 or 10·5.

For dose–response experiments 200, 400, 1000 or 2500 units of GM-CSF were injected i.p. in PBS. In some cases, GM-CSF was dissolved in medium RPMI 1640 before injection. For the experiments that were conducted in Canada with recombinant human GM-CSF (Genetic Institute) and murine GM-CSF (Genzyme), a single dose of 200 units was injected on Day 7·5 of pregnancy.

Spongiotrophoblast width. To estimate spongiotrophoblast width (Table 7), spongiotrophoblasts were sliced from fresh placentae with a surgical lance, then snap-frozen and measured by calipers in mm. The labyrinthine zone was also measured but the enlargement was not statistically significant in the limits of these experiments (number of placentae studied) and the data are therefore not shown.

Statistics. Student's *t* tests (placental width, fetal and placental weights) and χ^2 analysis (% resorptions) were performed to test the significance of treatment effects.

Results

Negative effects on pregnancy outcome

LPS from *Salmonella enteritidis* has been shown to induce complete fetal death in several strains of mice at a dose of 1 µg per mouse when given i.v. on Day 7·5 or Day 12·5 (a dose of 3 µg per mouse is required for abortion on Day 15–18) (Parand & Chedid, 1964), irrespective of the strain.

Table 1 shows typical experiments that confirm these data for various mating combinations of mice which are not abortion prone, as well as in the CBA × DBA/2 abortion-prone mice.

Direct measurement of TNF-α in the decidua confirmed that abortion was correlated with an enhancement of TNF levels (data not shown, and Chaouat *et al.*, 1990), in agreement with previous data (Parand & Chedid, 1964; Parand, 1987; Gendron *et al.*, 1989).

Table 1. Induction of fetal resorption (%, with no. viable, no. resorbing in parentheses) by lipopolysaccharide in mice of various mating combinations injected on Day 7, 12 or 15

Mating combination	Control mice	Treated mice*		
		1 µg i.v.		3 µg i.v.
		Day 7	Day 12	Day 15
C3H × BALB/c	6 (43, 2)	98 (1, 45)	100 (0, 48)	91 (4, 41)
C57BL/6 × DBA/2	6 (44, 3)	96 (1, 42)	98 (1, 39)	95 (2, 40)
C57BL/6 × BALB/c	8 (47, 4)	100 (0, 48)	96 (2, 53)	98 (1, 50)
CBA/J × BALB/c	10 (45, 4)	100 (0, 51)	100 (0, 48)	92 (4, 48)
CBA/J × DBA/2	38 (17, 28)	100 (0, 45)	100 (0, 49)	100 (0, 43)
C3H × DBA/2	5 (39, 2)	100 (0, 39)	100 (0, 40)	100 (0, 45)

*Mice were killed on Day 12 (injection on Day 7), Day 16 (injection on Day 12) or Day 19 (injection on Day 15).
All treatment groups showed significantly more resorptions than in the respective control groups, $P < 0.001$ (χ^2 tests).

When 1000 units of recombinant human TNF-α were injected on Day 7·5 i.p., a significant increase in fetal resorption rates was observed in CBA × DBA/2 mice as well as in the non-abortion-prone CBA × BALB/c and C3H × DBA/2 combinations. The effect was still significant when TNF-α was given on Days 9 and 10. However, to obtain a comparable level of fetal resorption rate, a higher dose of TNF was required on Days 13 and 17, although 1000 units significantly enhanced the fetal resorption rates above background levels (Table 2).

Poly(I).Poly(C12U), an inducer of γ-interferon production and an activator of natural killer cells (NKs) and lymphokine-activated killer cells (LAKCs), is known to increase fetal resorption rates (Baines & de Fougerolles, 1988; Chaouat *et al.*, 1988a, 1989a, b, c; R. Kinsky, G. Lepage,

Table 2. Induction of fetal resorptions (no. viable, no. resorbing) in mice by human recombinant TNF-α

Exp.	Mating combination	Control mice, % resorptions	Days of injection	% Resorption	Days of injection	% Resorption	Days of injection	% Resorption
					Treated			
1 (1000 Units)†	CBA × DBA/2	43 (12, 19)	7·5	79 (6, 22)*	—	—	—	—
	CBA × BALB/c	7 (26, 2)	7·5	89 (5, 39)*	—	—	—	—
	C3H × DBA/2	5 (34, 2)	7·5	47 (15, 17)*	—	—	—	—
2 (1000 Units)‡	CBA × DBA/2	36 (39, 22)	0·5	31 (43, 19)	3·5	83 (6, 30)*	—	—
			5·5	67 (22, 45)*	7·5	83 (11, 55)*	—	—
			9·5	88 (8, 58)*	10·5	75 (14, 43)*	—	—
			13·5	52 (35, 38)	17·5	47 (30, 27)	—	—
3	CBA × DBA/2	39 (33, 21)						
1000 Units†			7·5	89 (5, 41)*	13·5	60 (18, 26)*	17·5	51 (28, 29)
2000 Units†			7·5	100 (0, 42)*	13·5	94 (3, 45)*	17·5	57 (22, 29)
3000 Units†			7·5	100 (0, 48)*	13·5	59 (25, 36)*	17·5	85 (8, 45)*

†Killed Day 14·5.
‡Killed Day 12 (injections on Days 0·5–10·5), Day 17 (injections on Day 13·5) or Day 19·5 (injections on Day 17·5).
*$P < 0.05$ compared with control value for that Exp.

M. N. Thang & G. Chaouat, unpublished). We therefore also tested the effects of treatment with γ-interferon, and recombinant murine IL-2, a LAKCs and NK cell activator. Both treatments enhanced fetal resorption in the CBA × DBA/2 and CBA × BALB/c mating combinations (Table 3).

Table 3. Enhancement of fetal resorptions (%, no. viable, no. resorbing) by murine recombinant γ-interferon (1500 Units on Day 7·5) and murine recombinant IL-2 (2000 Units on Days 6·5, 8·5 and 10·5)

Mating combination	Control mice	Treated mice†	
		γ-Interferon	IL-2
CBA/J × DBA/2	38% (18, 11)	68% (8, 17)*	76% (8, 17)*
CBA/J × BALB/c	6% (31, 2)	44% (18, 14)*	55% (15/18)*

†Killed on Day 14.
*$P < 0.05$ compared with control.

Positive effects on pregnancy outcome

When IL-3 was injected i.p. fetal resorption rates were decreased in CBA × DBA/2 pregnancies whereas in the same experiment treatment with LPS or recombinant human TNF had the opposite effect (Table 4).

Table 4. Reduction of CBA × DBA/2 fetal resorption by murine IL-3 from WEHI cells

Exp.	Control mice			Treated mice†			
	% Resorbing	Placental wt (mg)	Fetal wt (mg)	Treatment	% Resorbing	Placental wt (mg)	Fetal wt (mg)
1	52 (14, 13)	102	121	IL-3‡	28 (15, 6)*	125*	141*
2	55 (17, 21)	—	—	IL-3†	22 (25, 7)*	—	—
3	46 (22, 19)	—	—	IL-3†	19 (30, 7)*	—	—
				LPS§	88 (3, 22)*	—	—
				H-rTNF-α¶	93 (2, 27)*	—	—
				PBS	54 (18, 21)	—	—

†Killed on Day 14·5.
‡600 Units on Days 6·5, 8·5 and 10·5.
§0·1 µg in PBS on Days 6·5, 8·5 and 10·5.
¶500 Units on Days 6·5, 8·5 and 10·5.
*$P < 0.05$ compared with controls.

Treatment with 200 or 400 units of purified natural GM-CSF decreased CBA × DBA/2 fetal resorption rates (Table 5). When different doses of GM-CSF were tested, dosages of > 1000 Units per injection were ineffective (Table 5). In fact, 3 × 2500 HCSF units sometimes enhanced fetal resorptions, although most often the enhancement was not significant, and at best P values were <0·02 (data not shown).

Treatment with recombinant murine GM-CSF (Genzyme) given on the same schedule at low doses was also protective (Table 6).

Both forms of murine GM-CSF not only affected fetal survival, but also enhanced placental (and fetal) weights (Tables 5 and 6). The enhancement appeared to depend markedly upon additional spongiotrophoblast width as a result of treatment (see Table 6). No significant size enhancement was traced in the labyrinth (data not shown).

Table 5. Reduction of CBA × DBA/2 fetal resorption (no. viable, no. resorbing) by purified murine GM-CSF from P338 D1

Exp.	Mating combination	Control mice			Treatment‡	Treated mice†		
		% Resorbing	Placental wt (mg)	Fetal wt (mg)		% Resorbing	Placental wt (mg)	Fetal wt (mg)
1	CBA × DBA/2	47 (27, 24)	95	118	GM-CSF400	8 (44, 4)*	136*	209*
	CBA × BALB/c	8 (37, 3)	138	211				
2	CBA × DBA/2	37 (17, 10)	—	—	PBS	51 (15, 6)	—	—
					GM-CSF200	7 (29, 2)*	—	—
3	CBA × DBA/2	38 (26, 16)	—	—	GM-CSF200	17 (38, 8)*	—	—
					GM-CSF400	24 (32, 10)*	—	—
					GM-CSF1000	32 (27, 13)	—	—
					GM-CSF2500	53 (18, 20)	—	—

†Killed on Day 14·5.
‡GM-CSF injected on Days 6·5, 8·5 and 10·5 at dose indicated.
*$P < 0.05$ compared with controls.

Table 6. Reduction of CBA × DBA/2 fetal resorption (no. viable, no. resorbing) by recombinant murine and human GM-CSF

Exp.	Mating combination	Control mice				Treatment‡	Treated mice†			
		% Resorbing	Plac. wt (mg)	Fetal wt (mg)	Spongio-trophoblast width (mm × 10^{-1})		% Resorbing	Plac. wt (mg)	Fetal wt (mg)	Spongio-trophoblast width (mm × 10^{-1})
1	CBA × DBA/2	52 (14, 13)	102	122	24·8	GM-CSF from P338 D1	24 (41, 13)*	140*	205*	33·5*
	CBA × BALB/c					R murine GM-CSF	30 (33, 14)*	133*	200*	33·1*
						R murine GM-CSF	17 (24, 5)*	141*	193*	30·6*
2	CBA × DBA/2	66 (11, 21)	—	—	—	R murine GM-CSF	10 (18, 2)*	—	—	—
	CBA × BALB/c	18 (29, 6)	—	—	—					
3	CBA × DBA/2	29 (43, 150)	—	—	—	Human R GM-CSF§	18 (32, 150)*	—	—	—
						R murine GM-CSF§	21 (51, 247)*	—	—	—

In Exps 1 and 2 mice were aged (see 'Methods'). Experiment 3 was carried out in Canada.
†Killed on Day 14·5.
‡On Days 6·5, 8·5 and 10·5.
§On Day 7·5.
*$P < 0.05$ compared with controls.

Similar experiments conducted in Canada used a recombinant human GM-CSF (Genetic Institute) and the same murine GM-CSF (Genzyme), but the control levels of resorption were much lower. A single dose of 200 units of either form of GM-CSF on Day 7·5 of pregnancy was minimally effective (Table 6). The murine GM-CSF stimulated placental cell growth *in vitro* but the human recombinant GM-CSF was inactive in this regard (data not shown, and Clark *et al.*, 1990). High doses of the human GM-CSF (10 000–30 000 units/mouse) were ineffective, similar to the data shown in Table 5.

Since P 338 D1 purified material contained traces of IL-1 and IL-3, it was important to ascertain that the effects of P 338 D1 supernatant and recombinant GM-CSF were mediated by GM-CSF itself and not by some contaminant in the preparation. Recombinant murine GM-CSF, or P 338 D1 material, was passed through a column containing normal rabbit serum coupled to CNBr-activated Sepharose 4B (Pharmacia, Pharmacia-LKB, Les Ulis, France) or similarly prepared rabbit anti-murine GM-CSF. The specific antiserum retained the fetal protective effect, while the control column eluant did not (Table 7).

Table 7. Removal of reduction of CBA × DBA/2 fetal resorption (no. viable, no. resorbing) by recombinant murine GM-CSF following adsorption with anti-GM-CSF antiserum coupled on Sepharose 4B

Mating combination	Control mice, % resorbing	Treated mice†	
		Treatment	% Resorbing
CBA × DBA/2	42 (30, 22)	GM-CSF	14 (47, 8)*
		GM-CSF + antiserum	38 (29, 18)
		GM-CSF on control column	24 (41, 13)*
CBA × BALB/c	7 (36, 3)*		

†Killed on Day 14·5.
*$P < 0.05$ compared with CBA × DBA/2 control.

Discussion

The results presented in this paper clearly demonstrate that pregnancy outcome can be positively or negatively regulated by injecting lymphokines directly into pregnant females. This raises the question of the mechanisms involved in this regulation. The trophoblast is resistant to cell-mediated lysis by normal killer cells (cytotoxic T lymphocytes, CTLs) or natural killer cells (NK), but is sensitive to lysis by lymphokine-activated killer cells (LAKC)s or CTLs obtained from a mixed lymphocyte reaction performed in a culture medium (Opti-MEM, Gibco) (a derivative of Minimal Essential Medium (MEM)) which is optimized especially for LAKCs culture generation, and which yields from a mixed lymphocyte culture/reaction CTLs endowed with LAKC-like killing activity (Gendron & Baines, 1988; Drake & Head, 1989). The peri-implantation embryo is surrounded by NK cells (Croy *et al.*, 1987) and some non-lymphoid cells at the implantation site, such as granulated metrial gland cells, have some NK-like activity, exerting cytostatic effects on 3·5-day blastocysts and 6·5-day embryonic tissue of mice (Croy & Kassouf, 1989). It is tempting to speculate that they could themselves be regulated by lymphokines, and secrete some lymphokines, and thus be involved in the control of placental growth (Wegmann *et al.*, 1989.

Furthermore, it has been shown that spontaneous abortion rates in the abortion-prone CBA × DBA/2 mouse system correlate with the local infiltration of asialo GM1-positive cells (Gendron & Baines, 1988). Indeed, it has been shown that embryos are first infiltrated by cells with NK-like activity, and only thereafter by some cytotoxic T lymphocytes (Chaouat, 1986). Trophoblast cells themselves are not sensitive to NK cell lysis, but human and mouse embryonic

fibroblasts, for example, are. Activated NKs and LAKCs are therefore a probable threat to fetal survival. It ensues that the local T cell activation/expansion by interleukin-2 and interleukin-4, -5 and -6, the activation/enhancement of NK lytic activity, also mostly by these lymphokines, and the differentiation and expansion in the decidua of LAKCs needs to be down-regulated at the feto–maternal interface for fetal survival. It is achieved in the decidua by decidual suppressor cells that secrete a TGF-β-2-like molecule (Clark *et al.*, 1988) and a placenta-derived factor which has immunosuppressive activity coupled to fibroblast growth factor properties, and other TGF-β-2-like properties (Chaouat *et al.*, 1990). (It is worth recalling that TGF-β 2 can block the proliferation/activation of the aforementioned lymphocyte subsets and, in addition, exert direct protective effects on targets of products from activated T and NK cells, exactly as does TNF, when tested on such targets as murine embryonic fibroblasts, or the murine malignant fibroblastic cell line, L929.) Although placenta-mediated suppression is deficient in resorbing animals on Day 11 (Chaouat *et al.*, 1985), no such difference is seen at earlier stages, whereas there is a clear correlation between the level of decidual suppression at the implantation site and subsequent fetal resorption (Clark *et al.*, 1987). It is therefore possible that a defect in local suppression would result in too high a level of local NK-cell activity, and recruitment and/or activation of LAKCs, thus leading to embryo demise.

Environmental factors are involved in the CBA × DBA/2 resorption models (Hamilton & Hamilton, 1987), most likely microbial in nature and probably resulting in local activation of non-specific effectors. IL-2 can lead to NK-cell differentiation into LAKCs, and it is likely that injections of excess IL-2 by-pass local active suppression and trigger such detrimental activation/differentiation. Our data are in keeping with those of Tezabwala *et al.* (1989), although in our hands we need to inject higher doses than they do to obtain induction of abortion. Such discrepancy is most probably due to environmental conditions, as discussed below for 'basal' resorption rates.

NK activation can be directly achieved in pregnant females by Poly(I).Poly(C12)U injection. Injection of spleen cells from animals Poly(I).Poly(C12)U into CBA/J females, or direct treatment of these, elicits abortion (Baines & de Fougerolles, 1988; Chaouat *et al.*, 1988c, d, 1989a, b). Such effects are seen not only in CBA/J, but also in BALB/c, C57BL/6 mice, which are non-resorption-prone strains (Chaouat *et al.*, 1989b, c, 1990; unpublished data). This paper demonstrates that the effects on pregnancy outcome of such activated NK cell-enriched cellular transfer can be mimicked by injecting TNF and γ-interferon, which are products of such activation. Their action could be cytostatic for placental cells, as demonstrated *in vitro*, or directly cytotoxic for non-trophoblastic components of the placenta (such an effect is highly likely in the case of embryonic fibroblasts), whereas IL-2 is likely to act by activating LAKCs themselves. In addition, TNF could act by causing local necrosis as a consequence of its action on the blood vessels that penetrate the placenta.

TNF could be produced as a result of local triggering of decidual T cells and NK cells, as well as macrophages, as a bystander effect of infection. LPS would merely mimic the infection-related event. Such a mechanism would account for environmental or ageing effects in the CBA/J × DBA/2 system (Hamilton & Hamilton, 1987; Chaouat *et al.*, 1986, 1988b). We took advantage of such effects to enhance the basal resorption rates in our CBA/J mice by letting them age for the R GM-CSF experiments. This effect accounts for most of the variation one can observe between our tables, since mice of the same age in the same room had no gross (statistically significant) variations in resorption rates throughout the year.

The effect of IL-3 is more complicated to explain. Such a therapeutic effect does not necessarily represent a physiologically significant event, because IL-3 has not yet been demonstrated at the maternal–fetal interface, although one report describes the presence of IL-3 mRNA in the decidua (Shorter *et al.*, 1989). Nevertheless, placental cells appear to be responsive *in vitro* to all members of the CSF family tested so far, and GM-CSF itself has been shown to have direct trophic effects on outgrowth of pure ectoplacental cone trophoblast (Armstrong & Chaouat, 1989).

GM-CSF can also be released from the decidua. The doses of recombinant murine GM-CSF that we inject are far too low to act directly on placental cell growth in the mouse, as deduced from in-vitro titration curves. Furthermore, human GM-CSF protects against high resorption rates in the murine CBA × DBA/2 matings. This lymphokine has no direct effect on murine placental cell growth *in vitro*, and human GM-CSF does not sustain the growth or promote colony formation by mouse progenitor cells. The involvement on placental or decidual cells of a new type of CSF receptor, able to mediate activities of this lymphokine on non-lymphoid progenitor, with no species restriction, has been suggested (G. Vadas, unpublished data) but not yet proved. The demon-stration of GM-CSF receptor on human trophoblast, possibly a new receptor, could clarify this issue (Uzumaki *et al.*, 1989). In the meantime, to explain these effects, we suggest at least four possible mechanisms, which are not necessarily mutually exclusive, and deserve consideration for future experimentation. (1) GM-CSF could trigger local release of CSF1 or more GM-CSF by macrophages or other cells leading to a direct or indirect trophic effect. (2) CSF1 might be released by placental cells themselves in response to low doses of GM-CSF in a paracrine loop, and high doses would result in a negative feed back. (3) Since some T cell lymphomas have been shown to secrete CSF1 (Pralloran *et al.*, 1989), and since HILDA/LIF, a product of CD4 and CD8 T cells has a direct trophic effect on extra somatic cells (Moreau *et al.*, 1988), the possibility that GM-CSF might have an effect on such a T cell subset is worthy of further examination. (4) GM-CSF is involved with interferon in natural suppressor mechanisms (Cleveland *et al.*, 1988), which share a certain number of properties with decidua and placenta-associated suppression, and IL-3 can abrogate LAKC activation/generation in the human (Gallagher *et al.*, 1988). Using the measure-ment of TNF-α to assess cytotoxicity of decidual supernatant on the L929 malignant murine fibroblast cell line, a classical test of TNF activity, we observed an inverse correlation in the CBA × DBA/2 system between TNF and GM-CSF titres (assessed as HCSF activity) in the decidua (Chaouat *et al.*, 1990). Although L929 is sensitive to TNF-α and -β, as well as the cytotoxic effects of other lymphokines, it is tempting to speculate that placental growth is under the control of decidual stop signals, amongst which TNF production by activated natural killer cells and lymphokine-activated killer cells, as well as granulated metrial gland cells, could be an element. Down-regulation by members of the CSF family of lymphokines of TNF production would result in enhanced placental growth, which would be functionally indistinguishable from a direct trophic effect, and may operate in parallel with it.

Whatever the mechanism(s) involved the results described in this paper indicate a direct influence of lymphokines on reproductive outcome *in vivo*.

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