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Mouse placental macrophages have a decreased ability to present antigen

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ABSTRACT **Large numbers of macrophages can be found in an animal's uteroplacental unit. This high concentration of macrophages suggests they must play an important role during placental development. To gain a better understanding of the functional capacity of placental macrophages, we have obtained a highly enriched placental macrophage culture and have derived several cell lines from this population. Both placental macrophages and cell lines show colony-stimulating factor 1-dependent growth, express Fc receptors, and can perform Fc-receptor-mediated phagocytosis. In addition, they express macrophage markers Mac-1, F4/80, and CD14. Although placental macrophages express major histocompatibility complex class II molecules constitutively, they display a decreased ability to present protein antigens to T cells. Since primary fetal liver macrophages of the same gestational stage also show a decreased ability to present antigens, this phenomenon may reflect a developmental stage of macrophages.**

Macrophages are found in the pregnant uterus and placenta (1, 2). These cells have been suggested to play many roles during pregnancy including the synthesis of monokines, such as tumor necrosis factor α (TNF-α) and interleukin 1 (IL-1) (2–4), the synthesis of immunosuppressive factors, and phagocytosis of debris and microbial invaders (2). In the mouse placenta, fetally derived macrophages contribute a significant portion of labyrinthine mesenchymal cells and cells of the chorionic villous stroma (2, 5). Macrophages have been detected by day 10 of pregnancy with significant numbers of morphologically matured macrophages, displaying the mononuclear-phagocytic-specific cell surface antigen F4/80 by day 12 (5). Although these placental macrophages were shown to be phagocytic, other functions remain to be demonstrated.

An important immunological function of macrophages is to activate T cells by serving as antigen-presenting cells and initiate an immune response (6). One previous report, however, has shown that placental macrophages are unable to initiate an efficient immune response against *Listeria monocytogenes* (7). To study the immunological function of placental macrophages, we have obtained a highly enriched primary placental macrophage population. In addition, we have isolated and characterized three macrophage cell lines from day 12 and 13 placentas. Our study shows that placental macrophages at this stage of development, despite expressing class II molecules, have a diminished ability to present protein antigens to T-cell hybridomas.

MATERIALS AND METHODS

Mice. BALB/c, C57BL/6, and A.CA mice were purchased from The Jackson Laboratory. BALB/k mice were provided by F. Lilly (Albert Einstein College of Medicine).

Reagents. Ovalbumin (OVA), keyhole limpet hemocyanin (KLH), cytochrome *c* (cyt*c*), hen egg lysozyme (HEL), and staphylococcal enterotoxin B (SEB) were purchased from Sigma. Trypsin-digested OVA and HEL fragments were generated by a published procedure (8). Anti-$A^{d/b}$(28.16.8s), anti-$E^{d/k}$(14.4.4s), anti-E^{k}(17.3.3s), anti-A^{k}(10-2.16), and F4/80-producing hybridomas were purchased from American Type Culture Collection. Anti-Mac-1 antibody was purchased from Boehringer Mannheim. Phycoerythrin (PE)-labeled goat anti-mouse IgM and IgG and fluorescein isothiocyanate-labeled goat anti-rat IgG were purchased from Caltag (South San Francisco, CA). Human IgG Fc fractions were purchased from Cappel Laboratories.

Cell Culture and Cloning. Cell lines 2.3 and SUPL2 were obtained from placentae dissected from 12 day (BALB/c × BALB/k)F_1 embryos. Placentae were cut into small pieces and cultured in colony-stimulating factor 1 (CSF-1)-containing growth medium. When outgrowing cells reached confluence, they were transformed with origin-defective simian virus 40 (SV40) DNA by the calcium phosphate precipitation method (9). Transformed cells were cloned by limiting dilution. JPL2A was derived from a placenta dissected from C57BL/6 mouse on day 13 of pregnancy and outgrowing placental cells were subcultured and cloned by limiting dilution 5–6 months later (10). The splenic macrophage cell line SC8 was obtained by transforming splenic macrophages of a (BALB/c × A.CA)F_1 mouse with origin-defective SV40 DNA (11). Peritoneal cells were harvested by peritoneal lavage from an unstimulated mouse. Primary placental and fetal liver macrophages and primary adult splenic macrophages were obtained by culturing appropriate tissue fragments in CSF-1-containing medium for a month.

Macrophage cell lines are grown in Dulbecco's modified Eagle's medium (DMEM; GIBCO) with 10% (vol/vol) fetal calf serum, 10% NCTC (Sigma), 1% penicillin and streptomycin, 1% glutamine, and 20% (vol/vol) L-cell conditioned medium as a source of CSF-1. A macrophage hybridoma, 63 (H-2^{d}–H-2^{k}), was a gift from M. Dorf (Harvard University) and was grown in DMEM with 10% fetal calf serum (12). T-cell hybridomas were gifts either from J. Kappler and P. Marrack (National Jewish Center, Denver) or from S. MacPhail (North Shore University Hospital). The IL-2-dependent CTLL-2 cell line was a gift from G. Ju (Hoffmann–La Roche) (11).

Assays. The lysozyme content in the supernatant of macrophage cultures was assayed as described (9). The ability of

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Abbreviations: MHC, major histocompatibility complex; PE, phycoerthytherin; CSF-1, colony-stimulating factor 1; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor α; IL, interleukin; IFN-γ, interferon γ; OVA, ovalbumin; KLH, keyhole limpet hemocyanin; cyt*c*, cytochrome *c*; HEL, hen egg lysozyme; SEB, staphylococcal enterotoxin B; SV40, simian virus 40; RPC, resident peritoneal macrophage.

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placental cells to secrete TNF-α and IL-1 after lipopolysaccharide (LPS; List Biological Laboratories, Campbell, CA) stimulation was determined as described (13, 14). The cathepsin D content of macrophage cell lysates was assayed as described (15). Fc-receptor expression and Fc-receptor-mediated phagocytosis were determined as described (9).

Antigen-Presenting Assay. Approximately 1×10^5 T-cell hybridomas cells were incubated with 1 to 5×10^4 macrophages and the appropriate antigen, enzyme-digested peptide fragments, or superantigen (SEB) in 200 μl for 24 h. Supernatants (75 μl) were transferred to another 96-well plate containing 5×10^3 CTLL-2 cells and assayed for IL-2 content by determining the incorporation of [^{3}H]thymidine by the IL-2-dependent CTLL-2 cells (11).

The Expression of Cell Surface Markers. Approximately 1×10^6 macrophage cells, preincubated with human IgG Fc fragments to block their Fc receptors, were incubated with 28.16.8s (anti-$A^{b/d}$), 10-2.16 (anti-A^k), 14.4.4s (anti-$E^{d/k}$), 17.3.3s (anti-E^k), anti-Mac1, F4/80, an irrelevant mouse IgG or IgM, or an irrelevant rat IgG on ice for 30 min, and then stained with PE-labeled goat anti-mouse IgG or IgM or goat anti-rat IgG. Fluorescence signals were measured and analyzed by EPICS-PROFILE II (Coulter).

DNA and RNA Analysis. Southern blot analysis of *Eco*RI (GIBCO/BRL)-digested genomic DNA was performed as described (16) and probed with a ^{32}P-labeled Eβ^d cDNA fragment (gift from D. R. Germain, National Institute of Allergy and Infectious Diseases, Bethesda, MD). Northern blot analysis of total cellular RNA was performed as described (16) and probed with a ^{32}P-labeled trophoblast-specific marker, A4311 cDNA fragment (17). The same blot was then stripped by boiling in 1× standard saline citrate (SSC) and probed again with ^{32}P-labeled murine CD14 cDNA fragment (18).

RESULTS

Analysis of the Antigen-Presenting Activity of Primary Placental Macrophages. There are three placental cell types, macrophages, decidual cells, and trophoblasts, that have receptors for CSF-1 and can potentially proliferate in response to CSF-1 (19). However, cells harvested after 1 month of culturing of 14-day placentae isolated from a (BALB/c × BALB/k)F_1 embryo in CSF-1-containing medium, express the macrophage-specific cell surface antigen detected with the antibody F4/80 and express major histocompatibility complex (MHC) class II molecules (Fig. 1). The level of MHC class II expression is similar to that of splenic macrophages (data not shown). This population was used as a source of primary placental macrophages. The antigen-presenting activity of primary placental macrophages was examined and compared to that of adult primary splenic macrophages. Table 1 shows that primary placental macrophages display a decreased ability to present whole protein antigens. However, they can present trypsin-digested OVA fragments to T cells. Since the majority of macrophages in the mouse placenta at this stage of gestation are of fetal origin (5, 20), the defect in antigen presentation we have observed may reflect the developmental stage of these macrophages. We therefore examined antigen presentation by macrophages derived from fetal liver of same gestational age obtained by the same method that was used for the generation of placental macrophages. Our results indicate that fetal liver macrophages are also deficient in their ability to present whole protein antigens (Table 1). To further analyze the antigen-presenting function of placental macrophages, we derived two cell lines, 2.3 and SUPL2, from primary placental macrophage cultures by transforming cells with an origin-defective SV40 DNA (9). In addition, we also derived a cell line, JPL2A, by spontaneous transformation of a primary placental cell culture of a different genetic background (10).

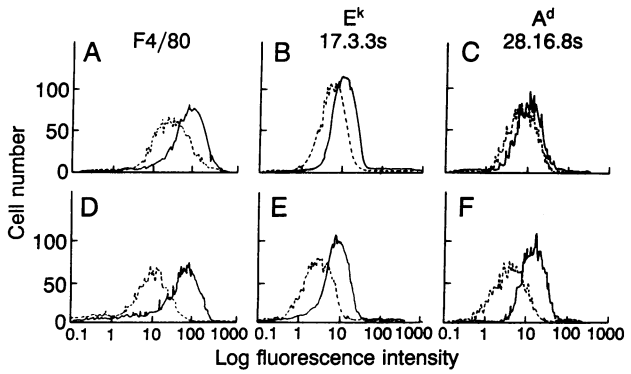

FIG. 1. Cell surface expression of the F4/80 antigen and MHC class II molecules of primary placental and fetal liver cells. Placental (*A–C*) or fetal liver (*D–F*) macrophages (1×10^6 cells), isolated from a 14-day (BALB/c × BALB/k)F_1 embryo and cultured for 1 month in the CSF-1-containing medium, were first incubated with human Fc fragments to block Fc receptor binding, washed, then incubated with irrelevant antibody (dashed line) or F4/80 (*A* and *D*), anti-E^k antibody (*B* and *E*), or anti-A^d antibody (*C* and *F*) (solid line) on ice for 1 h, washed again, and then incubated with PE-labeled goat anti-rat (*A* and *D*) or anti-mouse (*B*, *C*, *E*, and *F*) antibodies on ice for 30 min.

Characterization of the Cell Lines. RNA analyses showed that all three cell lines are negative for the trophoblast markers A4311 and cytokeratin but express vimentin (10) and the myeloid cell marker CD14 (21). In addition, all of the clones express the macrophage-specific F4/80 determinant and Mac-1 on their cell surface (10), and >99% of the cells of each clone express Fc receptors, can perform Fc-receptor-mediated phagocytosis, and secrete lysozyme. All the characteristics of these macrophage cell lines are summarized in Table 2. Since placental decidual cells do not express Mac-1 and F4/80 determinant, these results strongly suggest that these clones are not decidual cells but are of the macrophage lineage.

As the placenta contains cells of both maternal and fetal origin, we examined the origin of these cell lines. The 2.3 and SUPL2 cell lines were generated from a BALB/c female carrying a (BALB/c × BALB/k)F_1 fetus. The genotype of these clones can, therefore, be determined by the polymorphism of the class II Eβ gene (22). Southern blot analysis of *Eco*RI-digested 2.3 and SUPL2 genomic DNA, hybridized with Eβ cDNA probe, showed that both contain a 2.1-

Table 1. Primary placental and fetal liver macrophages have a decreased ability to present antigen

T-cell hybridoma	Antigen	[^{3}H]Thymidine incorporation, cpm		
		Spleen	Placenta	Fetal liver
DO11.10 (A^d)	—	939	1,805	0
	OVA	21,331	6,288	2883
	Peptides	14,504	18,144	7773
1E5 (E^d)	—	2,998	1,313	2821
	HEL	14,349	2,933	4018
DG11 (A^d)	—	14,972	449	126
2H10 (E^k)	—	195	268	514
	cytc	16,768	862	1330
SKK45.10 (A^k)	—	322	121	398
	KLH	5,827	428	292

Primary macrophages were obtained from the placenta and fetal liver of a 14-day (BALB/c × BALB/k)F_1 embryo and from an adult (BALB/c × BALB/k)F_1 spleen. Hybridoma DG11 (A^d) is an A^d-alloreactive T-cell hybridoma. Macrophage data are expressed as the mean of triplicate samples with an SD of <15% and represent at least three assays. Background cpm of T cells alone have been subtracted.

Table 2. Characterization of placental macrophage cell lines

Phenotype	2.3	SUPL2	JPL2A
CSF-1-dependent growth	+	+	+
Origin	Fetal	Fetal	ND
H-2 haplotype	d/k	d/k	b/b
Vimentin	+	+*	+*
A4311 mRNA	−	−*	−*
F4/80	+	+*	+*
Mac-1	+	+*	+*
CD14 mRNA	+	+	+
MHC class II	+	+	+
Fc receptor	+	+	+
Fc-mediated phagocytosis	+	+	+
Lysozyme	+	+	+
TNF-α†	+	+	+
IL-1†	+	+	−

ND, not determined; +, phenotype present; −, phenotype absent.
*See ref. 10.
†LPS-induced secretion.

kilobase band derived from Eβ^d gene and a 2.3-kilobase band derived from Eβ^k gene (Fig. 2). These data indicate that both 2.3 and SUPL2 are of fetal origin. Since the JPL2A cell line was generated from a C57BL/6 mouse, we cannot determine whether it is of fetal or maternal origin. Fig. 3 shows that 2.3 and SUPL2 express class II molecules of both d and k haplotypes. Although the A^k expression is relative low, 2.3 and SUPL2 express A^d molecules at a level similar to that of the splenic macrophage cell line SC8 also derived by SV40 transformation (data not shown). E^d-specific expression cannot be determined with the antibodies available. The JPL2A cell line has been shown (10) to express the A^b molecule.

Analysis of the Immunological Function of Placental Macrophage Lines. Since these macrophage cell lines are generated from a site of altered immune responsiveness, we examined their ability to secrete monokines upon stimulation and their ability to present antigens to helper-T-cell hybridomas. All three cell lines secrete >100 pg of TNF-α per million cells after 3 h of LPS stimulation. However, only 2.3 and SUPL2 cells, not JPL2A cells, can secrete >1500 units of IL-1 per million cells in response to LPS stimulation (Table 2). These placental macrophage cell lines show a decreased ability, relative to the splenic macrophage line SC8, the macrophage hybridoma 63, or peritoneal macrophages to present antigen to a panel of antigen-specific T-cell hybridomas and to an alloreactive T-cell hybridoma, 3DO62.1

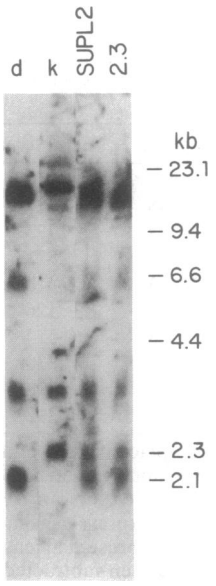

FIG. 2. Southern blot analysis of placental macrophages. Genomic DNA (20 μg), isolated from the BALB/c mouse (H-2^d; lane d), the BALB/k mouse (H-2^k; lane k), SUPL2 cells, and 2.3 cells, was digested with *Eco*RI and electrophoresed on a 0.8% agarose gel. After electrophoresis, DNA was transferred to a nylon membrane and hybridized with ^{32}P-labeled Eβ^d cDNA. kb, Kilobases.

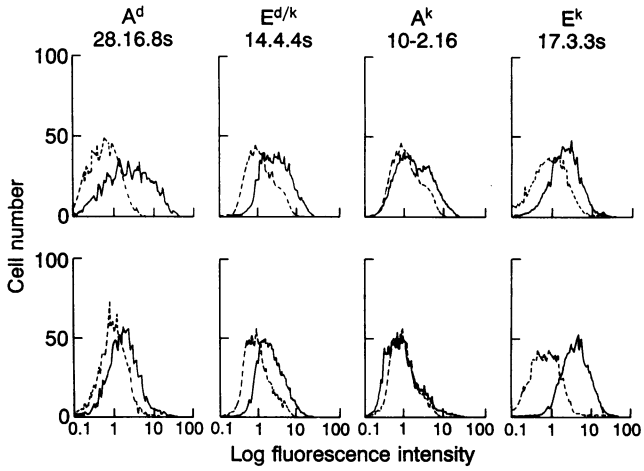

FIG. 3. Cell surface expression of class II molecules by placental macrophage cell lines. Approximately 1 × 10^6 macrophages were incubated with human Fc fragments to block Fc receptor binding, washed, then incubated with irrelevant antibody (dashed line) or anti-A or -E antibody (solid line) on ice for 1 h, washed again, and then incubated with PE-labeled goat anti-mouse IgG or IgM antibodies on ice for 30 min. (*Upper*) 2.3 cells. (*Lower*) SUPL2 cells.

(Table 3). Reduced antigen presentation by placental macrophages is not due to the release of any suppressive factor since the coculture of placental macrophages with splenic or peritoneal macrophages does not lead to decreased activation of T-cell hybridomas (data not shown).

Although 2.3 and SUPL2 have a decreased ability to present whole protein antigens, they do present trypsin-digested antigenic fragments to A^d- and A^k-restricted T-cell hybridomas (Table 4). This result suggests that they might have defects in their antigen-processing compartment. The DO11.10 T cell has been shown (23) to recognize an OVA fragment generated from a cathepsin D digest. We therefore assayed intracellular cathepsin D activity and found that SC8, 2.3, and SUPL2 have equivalent activity, 3.2 units, 6.8 units, and 4.0 units per million cells, respectively.

In contrast to 2.3 and SUPL2, JPL2A cells present neither OVA nor trypsin-digested OVA fragments to BO-97.10 T cells, which recognize these fragments presented on the surface of peritoneal macrophages of C57BL/6 origin. The addition of the IL-1 or peritoneal macrophages of the A.CA mouse, which by themselves cannot present OVA to BO-97.10 T cells, does not compensate this defect (Table 4).

We also examined the ability of lines 2.3 and SUPL2 to present superantigen, which does not require processing. Superantigens have been shown to bind class II molecules outside the antigen-binding groove (24). This interaction can then lead to the activation of a large number of T cells with T-cell receptors encoded by a single or restricted number of β chain variable region genes (25). Fig. 4 shows that both 2.3 and SUPL2 cells have a decreased ability to present the superantigen SEB to the SEB-reactive T-cell hybridoma DO11.10.

DISCUSSION

Fetal macrophages are among the earliest hemopoietic cells to be produced during development and they are relatively abundant in fetal tissue and placenta (20). To understand the function of placental macrophages, we have obtained a nearly homogeneous placental macrophage population and have also derived several cell lines from placental macrophage cultures. Phenotypic analysis indicated that all clones are macrophages, not decidual cells or trophoblasts. They all

Table 3. Placental macrophage cell lines display decreased antigen presentation

T cell	Antigen	[^{3}H]Thymidine incorporation, cpm					
		SC8	63	2.3	SUPL2	C57BL/6 RPC	JPL2A
DO11.10 (A^d/OVA)	−	851		0	108		
	+	52,396		12,896	2276		
3DO26.1 (E^d/OVA)	−	0		0	0		
	+	22,710		0	0		
AODK.16 (E^d/KLH)	−	59		5	0		
	+	10,046		0	0		
3DO62.1(E^d)	−	3,006		0	0		
1E5 (E^d/HEL)	−	1,630		431	316		
	+	43,219		2,906	1082		
2H10 (E^k/cytc)	−		138	64	71		
	+		11,480	395	552		
KK-4.5 (A^k/KLH)	−		202	24	42		
	+		36,748	711	692		
BDK-11.1 (A^b/KLH)	−					0	96
	+					15,987	11
BO-97.10 (A^b/OVA)	−					0	131
	+					20,402	122

SC8 is a SV40-transformed splenic macrophage cell line of the d/f haplotype. 63 is a macrophage hybridoma cell line of the d/k haplotype. See Table 1 for details. RPC, resident peritoneal macrophages.

possess many functions of macrophages generated from adult lymphoid organs.

Using the cytofluorimetric method, we are able to detect a low level of expression of class II antigens on placental macrophages. Previous studies, using immunohistological techniques, have reported the lack of class II antigen expression on macrophages at midtrimester gestational stage (26, 27). We believe this discrepancy represents the difference in sensitivity of the techniques used to identify class II molecules although it is possible that culturing the cells *in vitro* for 1 month induced class II expression. The level of class II expression is equivalent to that of a splenic macrophage cell line. The low binding of antibody 10-2.16 to 2.3 and SUPL2 cells is not due the lack of expression of A^k molecules, as 2.3 and SUPL2 can present trypsin-digested HEL fragments to an A^k-restricted T-cell hybridoma. Perhaps the A^k molecule expressed on placental macrophages assumes a conformation that is not recognized by the 10-2.16 antibody or perhaps the cells possess a density of A^k too low to detect by immunofluorescence but sufficient for T-cell activation. However, despite the expression of MHC class II molecules, primary placental macrophages and placental macrophage cell lines exhibit a decreased ability to present whole protein antigens to T-cell hybridomas. This phenomenon may result from an alteration in accessory molecules or a modification in the antigen-processing compartment. Since placental macrophages can present trypsin-digested fragments to T cells, the decreased ability to present whole protein antigens is likely due to an alteration in the antigen-processing pathway. Furthermore, the lack of antigen processing is not solely due to a deficiency in proteolytic enzymes, as 2.3 and SUPL2 cells contain adequate levels of cathepsin D activity, but rather may reflect some alteration in endosomal function preventing proteolysis of engulfed antigen or impeding the association of peptide fragments with the class II molecule. The additional observation that 2.3 and SUPL2 cells have a diminished capacity to present superantigens, which do not require processing, suggests that placental macrophages may have altered their antigen-presenting pathway at several stages, such as modifications in their class II molecules or in some as yet undefined accessory molecule needed for antigen presentation. Since macrophages derived from fetal liver at

Table 4. Some placental macrophage cell lines can present antigenic peptides

Exp.	APC	Treatment	Trypsin-digested peptide	[^{3}H]Thymidine incorporation, cpm	
				− peptide	+ peptide
A	SC8	—	OVA	851	52,076
	2.3		OVA	0	56,519
	SUPL2		OVA	108	33,923
B	(BALB/c × BALB/k) RPCs	—	HEL	25	31,863
	2.3		HEL	30	56,364
C	C57BL/6 RPCs	Alone	OVA	71	16,927
		A.CA RPCs	OVA	2893	16,691
		Alone	OVA	43	25,224
		IL-1	OVA	43	22,322
	JPL2A	Alone	OVA	0	645
		A.CA RPCs	OVA	16	265
		Alone	OVA	0	1,249
		IL-1	OVA	0	248

Experiments: A, DO11.10 T cells—an E^d-restricted and OVA-specific T-cell hybridoma; B, KHEL25.14 T cells—an A^k-restricted and HEL-specific T-cell hybridoma; C, BO97.10 T cells—an A^b-restricted and OVA-specific T-cell hybridoma (for details, see Table 1). OVA at 1 mg/ml, HEL at 0.25 mg/ml, and trypsin-digested fragments at 0.25 mg/ml were used. APC, antigen-presenting cell.

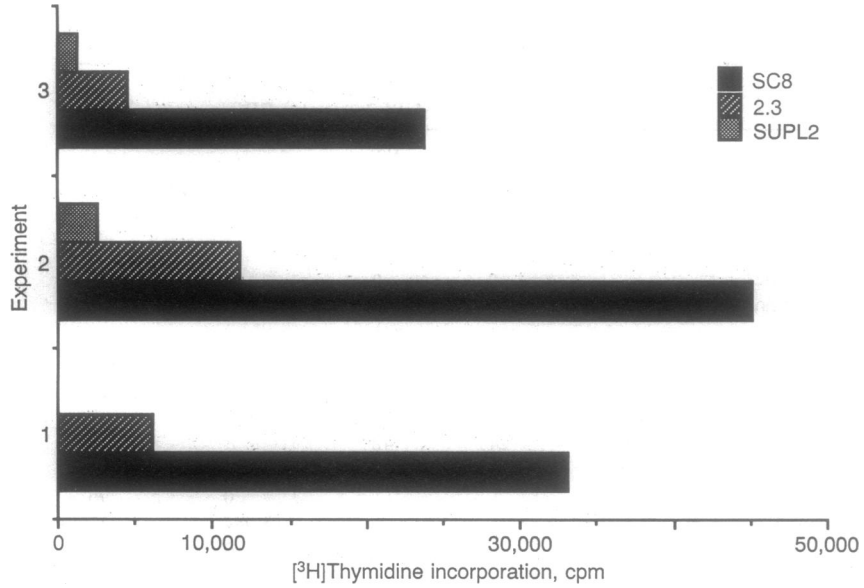

FIG. 4. Presentation of SEB by a splenic macrophage cell line, SC8, and placental macrophage cell lines 2.3 and SUPL2. Approximately 1×10^4 macrophages and 1×10^5 DO11.10 T cell hybridomas were incubated with SEB at 5 μg/ml in 200 μl of medium. IL-2 secretion by DO11.10 T cells after 24 h was measured. Data from three experiments are presented.

the same gestational stage have a similar deficit in antigen presentation, this phenomenon may be a property of fetal macrophages and a consequence of their developmental stage.

JPL2A cells, on the other hand, have defects in presenting both whole and enzymatically digested protein antigens. This defect is not due to the lack of IL-1 secretion or any other costimulatory factors (28). This result suggests that there may be a functional heterogeneity among primary placental cells, and this heterogeneity probably is not detected in the bulk placental culture.

The mechanism for protecting the fetus from maternal immunological rejection is likely to be multifactorial, including humoral and cellular factors. Fetal liver γ/δ^+ T cells that exhibit cytotoxic activity toward maternal T cells have been identified, and these cells may function to destroy the invading maternal T cells (29). Our results imply that the decreased antigen presentation of placental/fetal macrophages may serve as another mechanism to prevent the allogeneic response of maternal T cells and may also explain the lack of macrophage bactericidal activity during uteroplacental infection (7). These placental macrophage cell lines provide a useful system to study antigen processing at the molecular and cellular levels.

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