APOPROTEIN E IS SYNTHESIZED AND SECRETED BY RESIDENT AND THIOGLYCOLLATE-ELICITED MACROPHAGES BUT NOT BY PYRAN COPOLYMER- OR BACILLUS CALMETTE-GUERIN–ACTIVATED MACROPHAGES*

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Macrophages exist in a variety of functional and morphologic states in tissues: resident quiescent cells, inflammatory macrophages stimulated by sterile chemical stimulators, and macrophages activated by microorganisms and immunological stimuli. A number of biochemical and functional criteria that distinguish between these phenotypic states have been assembled (1–3). These include ectoenzyme markers such as 5′-nucleotidase, which is present on resident macrophages but not on elicited macrophages (4), the enhanced secretion of lysosomal and proteolytic enzymes by chemically elicited inflammatory and activated macrophages (5–8), and the microbicidal and cytostatic properties of activated macrophages (9–12). Libraries of monoclonal antibodies against surface constituents have begun to reveal heterogeneity in inflammatory macrophage populations (13, 14).

Approximately 10% of the newly synthesized proteins of macrophages are secreted into the extracellular milieu of these cells (15, 16). A macrophage secretes more than 60 polypeptides (15, 16), including apoprotein E (17, 18), fibronectin (19), complement factor B (20), plasminogen activator (21, 22), and other proteinases (22, 23). Secreted proteins are particularly sensitive to alteration in response to inflammation (24). Because there are differences in the secretion of neutral proteinases by macrophages in different functional states (7, 8, 21, 22), we have investigated whether the secretion of other proteins varies according to the quiescent, activated, or inflammatory state of the macrophage. In this paper, we report that apoprotein E (ApoE),[1] a protein of M_r 33,000 that is the constituent of plasma lipoproteins involved in receptor-mediated clearance of lipids from the circulation (25) and in immune regulation (26, 27), is synthesized by resident macrophages and by inflammatory macrophages elicited with nonspecific stimuli such as thioglycollate broth, but is not synthesized by the

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[1] *Abbreviations used in this paper:* ApoE, apoprotein E (also called apolipoprotein E or arginine-rich apolipoprotein); BCG, bacillus Calmette-Guerin; DME, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; VLDL, very low density lipoprotein.

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hydrogen peroxide-producing, immunologically activated macrophages elicited in mice infected with living *Mycobacterium bovis* strain bacillus Calmette-Guerin (BCG), or injected with pyran copolymer, killed *Corynebacterium parvum*, and endotoxin.

Materials and Methods

Antisera. Rabbit antibody to rat ApoE, purified rat ApoE, and ^{125}I-labeled mouse very low density lipoproteins (VLDL) were provided by Dr. Thomas Innerarity, Gladstone Foundation Laboratories, University of California, San Francisco. Mouse VLDL was isolated and iodinated (28), and rat ApoE was isolated and rabbit antibody to it was produced as described previously (29). The IgG fractions from the rabbit anti-rat ApoE and from nonimmune rabbit serum were prepared by affinity chromatography on protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ). Rhodamine- or horseradish peroxidase-labeled IgG fractions of sheep anti-rabbit IgG was purchased from Cappel Laboratories, Cochranville, PA.

Resident and Inflammatory Macrophages. Resident and inflammatory macrophages were obtained from the peritoneal cavity of outbred Swiss-Webster CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA). In some experiments endotoxin-resistant C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) and the coisogenic endotoxin-sensitive C3H/HeN strain (Charles River Breeding Laboratories) were used as a source of macrophages.

Thioglycollate-elicited macrophages (6, 7) were obtained 4 d after intraperitoneal injection of 1 ml of aged 3% Brewer's thioglycollate broth. BCG-activated macrophages (13, 21) were obtained 10–12 d after intraperitoneal injection of 2–6.5 × 10^6 viable BCG organisms (tuberculin vaccine; Glaxo Inc., Fort Lauderdale, FL); alternatively, boosted BCG-activated macrophages were obtained after an injection of live BCG followed 14 d later by 1 mg of heat-killed BCG (cell walls from *M. bovis*, lot RIR201; RIBI ImmunoChem, Inc., Hamilton, MT). Pyran copolymer-activated macrophages (7, 30) were obtained 4 d after the injection of 25 mg/kg of pyran copolymer (gift of Hercules Chemical Co. Inc., New York). *Corynebacterium parvum*-activated macrophages (31, 32) were obtained 4–10 d after intraperitoneal injection of 1 mg of heat-killed *C. parvum* (whole cells, *P. acnes*, strain 4182, lot RIR-551; RIBI ImmunoChem, Inc.); macrophages were also obtained after injection of 1 mg of the pyridine extract of *C. parvum* (*C. parvum* PE, *P. acnes* strain 4182, lot RIR-401; RIBI ImmunoChem, Inc.) (32). Endotoxin-activated macrophages (7) were obtained 4 d after intraperitoneal injection of 10 μg of detoxified endotoxin from *Salmonella typhimurium* (lot RIR-353; RIBI ImmunoChem, Inc.). Sodium metaperiodate-elicited macrophages were obtained 4 d after the injection of 0.5 ml of 5 mM $NaIO_4$ (7). Each inflammatory agent was used in at least four independent experiments.

To test the effects of inflammatory agents on resident peritoneal macrophages in the absence of cells newly recruited from blood and bone marrow, macrophages were lavaged from mice treated with the glucocorticoid dexamethasone or with x radiation. Mice were treated with daily subcutaneous injections of dexamethasone (decadron phosphate; Merck Sharpe & Dohme, West Point, PA), 400 mg/kg for 3 d (33), and peritoneal macrophages were lavaged at 4 d. In the alternative protocol, mice were irradiated with 2.5–5.0 Gy (1 Gy = 1 J/kg = 100 rad) of x rays (34), the inflammatory agents were injected intraperitoneally 24 h later, and peritoneal cells were lavaged 3 d later.

Peritoneal cells were explanted in culture for 2 h in Dulbecco's modified Eagle's medium (DME) containing 10% fetal bovine serum (FBS). Nonadherent cells were removed by washing and the macrophages were either radiolabeled immediately or placed in medium with 10% FBS for various lengths of time. In some experiments, the nonadherent cells were labeled in suspension. To test for alterations in secretion induced in macrophages by adherence, cells from the total peritoneal exudate were labeled in suspension immediately after lavage.

The proliferative capacity of peritoneal macrophages was determined by plating peritoneal macrophages in DME containing 10% FBS, 10% horse serum, and 10% L cell-

conditioned medium at 1×10^4/4-cm^2 dish (6, 35). High clonigenic capacity was rated at >50 colonies per dish, and low clonigenic capacity as <5 colonies per dish.

Biosynthetic Labeling of Macrophage Proteins and Gel Electrophoresis. Macrophages (usually 5×10^5/4-cm^2 culture well) were incubated for varying times with 25 μCi/ml [^{35}S]-methionine (>1,000 Ci/mmol; Amersham Corp., Arlington Heights, IL) in methionine-free DME. Radiolabeled cellular and secretory protein samples were then prepared and separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis on 7–15% gradient gels, and fluorographs were prepared as described previously (15). The patterns of secreted proteins changed with labeling time in culture >4–8 h because of maturation of the macrophage and/or proteolytic processing; a 2-h labeling period was determined to be the optimal time to observe both cellular and secreted proteins and was used for all experiments unless indicated otherwise. Typically, 5×10^5 macrophages incorporated $5–10 \times 10^5$ dpm of [^{35}S]methionine into cellular proteins and $2–10 \times 10^4$ dpm into secreted proteins in 2 h. ^{14}C-labeled proteins used as molecular weight standards were filamin, myosin, macroglobulin, phosphorylase B, bovine serum albumin, heavy and light chains of IgG, ovalbumin, carbonic anhydrase, lactoglobulin A, lysozyme, and cytochrome C (Amersham Corp.). Samples loaded on gels contained aliquots of cellular and secreted proteins from either identical numbers of cells or identical amounts of radioactivity from preparations. Amounts of radiolabeled ApoE and other proteins were determined by densitometry of the fluorographs or by excising bands from gels and counting radioactivity in a liquid scintillation spectrometer.

Immunoprecipitation of ApoE. IgG from a specific rabbit anti-rat ApoE serum gave a single line of identity in Ouchterlony double diffusion gels between rat serum, mouse serum, purified rat ApoE, and mouse VLDL, and gave a single ring of precipitation by radial diffusion (not shown). For immunoprecipitation of [^{35}S]methionine-radiolabeled macrophage secretion products, either specific immune IgG from rabbit anti-rat ApoE or control IgG from rabbit anti-bovine serum albumin (Cappel Laboratories) was incubated with the macrophage secretion products. The immune complexes were then removed by means of formalin-fixed *Staphylococcus aureus* Cowan I strain, which has protein A on its surface (Zysorbin; Zymed Laboratories, South San Francisco, CA) as described previously (36). For comparison, ^{125}I-labeled mouse VLDL from mouse plasma or ^{125}I-labeled rat ApoE was also electrophoresed on gels. Fibronectin (M_r 240,000) present in macrophage secretions also bound to *S. aureus* cell walls (37, 38) and was also seen in both immune and control immunoprecipitations.

ApoE in macrophage lysates or secretions was also investigated by an immunoblotting procedure (39). Proteins from 2×10^6 cells or their secretions separated by SDS-polyacrylamide gel electrophoresis were transferred onto nitrocellulose filters incubated for 3–4 h with rabbit anti-ApoE IgG (5 μg/ml), washed, incubated with horseradish peroxidase-labeled sheep anti-rabbit IgG (1 μg/ml) for 30 min, washed, and then stained. Parallel gels were stained for total protein with Coomassie blue R250.

Partial Proteolytic Peptide Maps of ApoE Secreted by Macrophages. [^{35}S]methionine-labeled ApoE that had been separated by SDS-polyacrylamide gel electrophoresis and identified by autoradiography was excised from dried gels, then partially digested with chymotrypsin; the peptides were mapped on a 15% SDS-polyacrylamide gel as previously described (40). The ^{125}I-labeled ApoE band from mouse VLDL was mapped for comparison.

Immunofluorescence. To localize ApoE in secreting macrophages, cells were plated on glass coverslips at 5×10^4/cm^2 in DME plus 10% FBS for various lengths of time. Cells were then washed three times with phosphate-buffered saline (PBS) and fixed for 10 min at room temperature in 2% formaldehyde (freshly prepared from paraformaldehyde) in PBS. The fixed cells were washed with 50 mM NH_4Cl in PBS for 10 min to quench any remaining aldehyde functions (41), two times with PBS, and once with 3% sheep serum in PBS. They were then made permeable by incubation in 0.1% Triton X-100 for 10 min, washed two times in PBS, and once with 3% sheep serum. Cells were then incubated with rabbit anti-rat ApoE IgG at 10–100 μg/ml in 3% sheep serum for 45 min. The coverslips were washed two times in PBS and once in 3% sheep serum. The cells were incubated with the second antibody, rhodamine-labeled IgG from sheep anti-rabbit IgG,

at a 1:100 dilution in sheep serum for 45 min at room temperature. The cells were then washed three times and the coverslips were examined in the wet state in PBS with a water-immersion lens, or mounted in polyvinyl alcohol (Gelvatol; Monsanto Co., St. Louis, MO) (42), and examined with a water or oil immersion lens using a Zeiss Photomicroscope III (Carl Zeiss, Inc., Thornwood, NY) equipped with epifluorescence optics. The Gelvatol-mounted samples lost some of their contrast when observed with phase-contrast or Nomarski optics, but the immunofluorescence was stable indefinitely at room temperature. The results were photographed on Kodak Tri-X film, rated at 400 ASA, or on Ektachrome 400, for exposures of 15–60 s. The controls used for the immunofluorescence included (*a*) nonimmune rabbit IgG instead of the rabbit anti-rat ApoE IgG, (*b*) anti-ApoE IgG adsorbed out with an excess of rat ApoE or rat serum, and (*c*) both permeable and nonpermeable macrophages.

Biochemical Assays. H_2O_2 production, triggered by the addition of 12-*O*-tetradecanoylphorbol-13-acetate (50 ng/ml), was assayed by the peroxidase-mediated extinction of scopoletin fluorescence (9, 43–45). Adherent cells were incubated in 12- or 24-well plates in RPMI-1640 (without phenol red) containing 10 mM Hepes, pH 7.4, 20 μM scopoletin, and 1 μM horseradish peroxidase at 37°C for various times up to 60 min. Control cells were incubated with 2,000 U/ml catalase. After incubation the medium was removed and centrifuged for 3 min at 9,000 *g* to remove cells. The scopoletin fluorescence at 350 nm excitation and 460 nm emission was recorded. The change in fluorescence relative to that of medium incubated without cells was calculated and corrected for the non–catalase-suppressible change in fluorescence. As determined morphologically, the adherent populations contained 0–8% polymorphonuclear leukocytes, depending on the stimulus and time in culture. Results were corrected for contamination of adherent inflammatory macrophage cultures by polymorphonuclear leukocytes.

Elastase and plasminogen activator present in the conditioned medium of macrophage cultures were assayed as described previously (6, 7). 5′-Nucleotidase in Triton X-100 lysates of macrophages was assayed with 5′-[^{3}H]AMP as substrate (46). β-Glucuronidase was assayed using methylumbelliferyl-β-glucuronide as substrate (47).

Results

ApoE Is the Major M_r 33,000 Secretory Protein of Thioglycollate-elicited Macrophages. When the conditioned medium from thioglycollate-elicited macrophages labeled with [^{35}S]methionine was displayed on an SDS-polyacrylamide gel, a number of major polypeptides were visible, including peptides of M_r 240,000, 93,000, 60,000, and 33,000 (Fig. 1 *a* and *b*). Rabbit anti-rat ApoE IgG immunoprecipitated the major band at M_r 33,000 and minor bands at M_r 35,000. These species arise from variable glycosylation of ApoE (18). ^{125}I-labeled ApoE from mouse VLDL that was immunoprecipitated under identical conditions displayed a major component at M_r 33,000 and minor bands. These bands were identical, as determined by mapping of their digestion by chymotrypsin (Fig. 1 *c*). The ApoE band was readily visible on silver-stained gels of 24–48-h conditioned medium from the secreting macrophages, and after 10-fold concentration it was comparable by immunodiffusion to the ApoE concentration of normal mouse serum (~50 μg/ml), suggesting that, for 10^6 cells, concentrations of 1–10 μg ApoE/ml accumulated in the medium (not shown). When the radiolabeled conditioned medium protein was allowed to incubate with macrophages for 24–48 h, additional lower molecular weight bands, identified as being derived from ApoE by immunoprecipitation and peptide mapping, appeared owing to proteolysis by secreted proteinases of the macrophage (see also Fig. 4 *a*).

Because the metabolic function of macrophages changes with adherence (48), thioglycollate-elicited macrophages were labeled in suspension immediately after

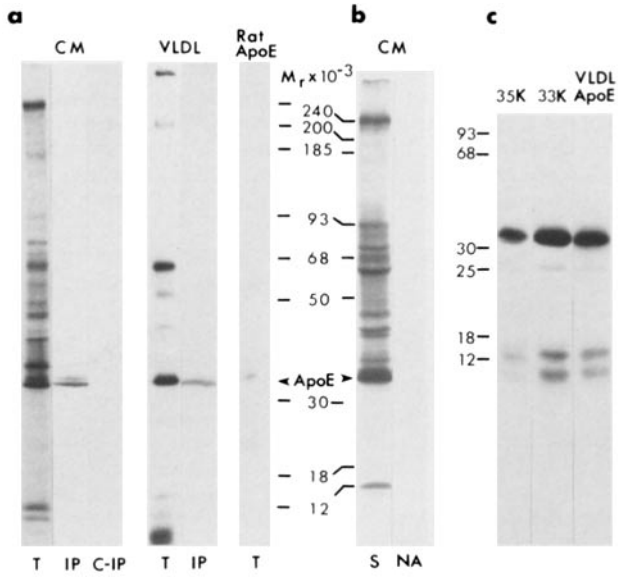


FIGURE 1. Secretion of ApoE by thioglycollate-elicited macrophages. (*a*) Immunoprecipitation of ApoE from conditioned medium (CM) of thioglycollate-elicited macrophages or from VLDL proteins. CM lane: T, total acid-precipitable secreted proteins; IP, proteins immunoprecipitated with anti-ApoE IgG; C-IP, proteins immunoprecipitated with anti-bovine serum albumin IgG. VLDL lane: T, total ^{125}I-labeled mouse VLDL proteins; IP, VLDL proteins immunoprecipitated with anti-ApoE IgG. Rat ApoE lane shows ^{125}I-labeled purified rat ApoE, which migrates at a higher M_r than mouse ApoE. (*b*) Secreted proteins in conditioned medium of thioglycollate-elicited peritoneal exudate radiolabeled with [^{35}S]methionine in suspension (S) compared with secreted proteins of the nonadherent lymphocyte population (NA) from the same cells. See secreted proteins (T) of adherent thioglycollate-elicited macrophages in *a* for comparison. (*c*) Partial chymotryptic peptide maps of immunoprecipitated ApoE from minor M_r 35,000 (35 K) band from macrophage CM, major M_r 33,000 (33 K) band from macrophage CM, and major M_r 33,000 band from mouse VLDL (VLDL ApoE). M_r ($\times 10^{-3}$) markers and migration of ApoE are indicated.

lavage from the peritoneal cavity. Adherence was not required for secretion of ApoE by macrophages. The nonadherent lymphocytic subpopulation of cells secreted no detectable ApoE (Fig. 1 *b*).

ApoE secreted into the medium was first detected after a lag period of 15–30 min, reaching a constant rate after 1 h of continuous labeling. The secretion of radiolabeled proteins was linear for at least 8 h (Table I). After a 15-min pulse-labeling period, when little ApoE had appeared extracellularly, the ApoE was a distinct intracellular band representing ~1% of the total labeled protein. Within a 60-min chase, virtually all of the secretory protein appeared in the medium, as had been observed previously (49). After 1–2 h of labeling, the total labeled intracellular ApoE had reached a constant, while its percentage of total labeled protein continued to decrease until 24 h. The secreted ApoE increased in the culture medium for 24 h, and its relative concentration remained constant between 1 and 8 h of labeling. Because of the rapid onset of changes in total secretion occurring within the first 24 h after explanting macrophages into culture (see below), a 2-h pulse-labeling period was chosen as optimal for determining the intracellular and secretion patterns that most closely resembled

TABLE I

*Incorporation of [^{35}S]Methionine into Cellular and Secreted Proteins of Thioglycollate-elicited Macrophages During Continuous Labeling**

Labeling time	Cellular proteins		Secreted proteins	
	Total	ApoE‡	Total	ApoE‡
h	*dpm* × 10^{-4}		*dpm* × 10^{-4}	
0.5	109	0.6	1.6	0.2
1.0	189	0.8	3.0	0.6
2.0	391	0.9	7.6	1.1
4.0	711	1.0	12.9	1.9
7.5	1,770	1.7	39.2	3.2
24.0	3,522	1.5	127.0	16.5

* Macrophages (5×10^5) were cultured for 2 h, then labeled with [^{35}S]-methionine for the times indicated.

‡ Determined by immunoprecipitation.

the phenotype of the macrophages in vivo, and was used in all the rest of the experiments described in this paper.

Macrophages Display Distinct Phenotypes in Secretion of ApoE. We next compared the relative and absolute rates of ApoE secretion by resident and elicited peritoneal macrophages 2 h after lavage. Immediately after explanting in culture, the macrophages derived from mice that had received various inflammatory stimuli displayed patterns of secreted proteins that were markedly different from each other (Fig. 2 *a* and *b*), whereas their cellular proteins had fewer differences (Fig. 2 *c*). Resident macrophages and macrophages elicited by thioglycollate broth secreted ApoE at high rates, and $NaIO_4$-elicited macrophages secreted at intermediate rates. However, macrophages obtained from animals infected with BCG or treated with pyran copolymer, whole *C. parvum*, or endotoxin had depressed total rates of protein secretion and secreted ApoE at $<1/10$ the rate of resident macrophages (Fig. 2, Table II). Macrophages elicited with the pyridine extract of *C. parvum*, which retains some of the properties of the whole organism but is unable to induce tumor-suppressing activated macrophages (31, 32), secreted ApoE at a high rate (Table II). ApoE was not specifically suppressed in the activated inflammatory macrophages obtained from the BCG-, *C. parvum*-, and endotoxin-treated animals. A variable number of other proteins such as fibronectin were suppressed with some treatments, whereas other polypeptides, such as lysozyme (M_r 14,000) and complement factor B (M_r 93,000) (20 and unpublished observations), changed relatively little.

Activated Macrophages Initiate ApoE Secretion with Time in Culture. Within 24–48 h in culture, both the rate of secretion and the pattern of secreted proteins from the inflammatory macrophages had changed markedly (Fig. 2). Although the resident and thioglycollate-elicited macrophages continued to secrete ApoE in the same relative proportions (Fig. 2, Table II), the total secretion of proteins was stimulated considerably, increasing up to fivefold by 7 d (Fig. 3). At 2 h in culture, BCG-, pyran copolymer-, *C. parvum*-, and endotoxin-activated macrophages secreted ApoE at $<1/7$ the rate of resident and thioglycollate-elicited macrophages (Fig. 3, Table II). The time of onset of ApoE secretion by BCG-

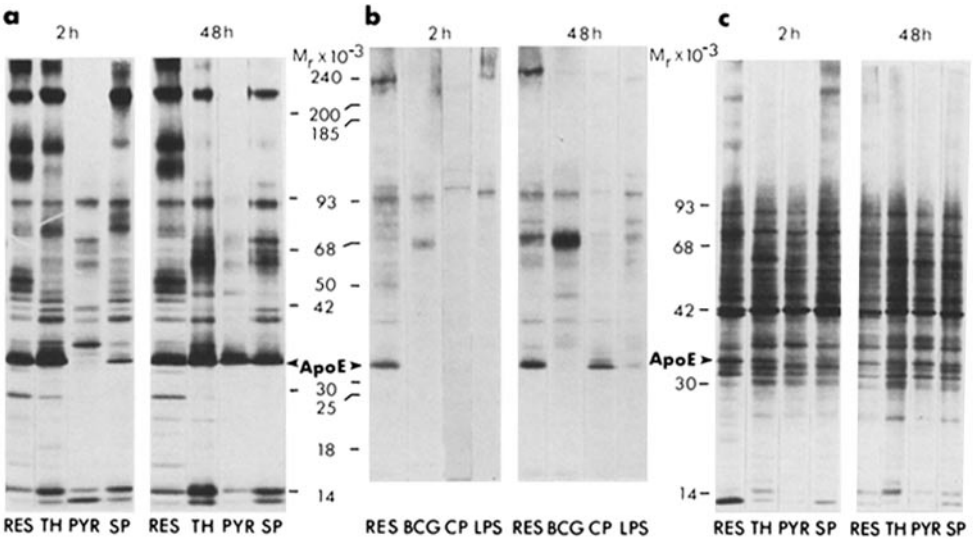


FIGURE 2. Secreted (*a* and *b*) and cell-associated (*c*) proteins of resident macrophages and macrophages elicited by inflammatory agents radiolabeled with [^{35}S]methionine for 2 h, at 2 or 48 h after explanting into culture. Results are from two different experiments. RES, resident; TH, thioglycollate elicited; PYR, pyran copolymer elicited; SP, $NaIO_4$ elicited; LPS, endotoxin elicited; CP, whole *C. parvum* elicited; BCG, BCG elicited. In the experiment shown in *b*, lysozyme (M_r 13,000) migrated at the buffer front and is not shown. M_r ($\times 10^{-3}$) markers and migration of ApoE are indicated. Aliquots containing 1.5×10^4 cpm were applied to each lane of the gel. Fluorographs were exposed for 5 d.

activated macrophages was somewhat variable. Macrophages obtained after one injection with living organisms initiated ApoE secretion after 24–72 h in culture, and its secretion as well as total protein secretion increased progressively with time to 7 d in culture in medium containing serum (Fig. 3, Table II). Macrophages from BCG-infected animals boosted with killed BCG initiated ApoE secretion after 48–96 h in culture (Fig. 2); this variable onset may have been due to variable activation of the macrophages, to variable numbers of activated lymphocytes remaining in the cultures, or to the persistence of bacterial products in the cells. Secretion of ApoE and other proteins was also induced in the *C. parvum*-, endotoxin-, and pyran copolymer-activated macrophages by 24–48 h (Figs. 2 and 3, Table II).

Intracellular ApoE in Macrophages Parallels Secretion of ApoE. We next investigated whether the macrophages that did not secrete radiolabeled ApoE during the first 2 h in culture contained intracellular ApoE. By immunoblotting, freshly lavaged thioglycollate-elicited macrophages contained intracellular ApoE in amounts approximately equivalent to that secreted in 2–4 h, whereas pyran copolymer-activated macrophages contained no detectable ApoE and did not release unlabeled ApoE into the medium during their first few hours in culture (Fig. 4*a*). By 24 h in culture, immunoreactive ApoE appeared in the pyran copolymer-elicited macrophages and their secretions (Fig. 4*a*). By immunoprecipitation, ApoE was determined to be ~0.5–1% of the intracellular protein newly synthesized in 2 h by freshly lavaged thioglycollate-elicited macrophages but was not detected in pyran-activated macrophages (Fig. 4*b*). After 48 h in

TABLE II

*Effect of Inflammatory Stimuli on Secreted and Intracellular ApoE of Macrophages After 2 or 48 h in Culture**

Stimulus	[^{35}S]methionine incorporated into proteins (2 h, per 10^6 cells)				Secreted ApoE (percent of total secreted protein)		Intracellular ApoE (percent ApoE-positive cells)	
	Total		Secreted		2 h	48 h	2 h	48 h
	2 h	48 h	2 h	48 h				
	dpm × 10^{-4}							
None	87	114	7.6	10.9	8.8 (7.2–26.2)‡	7.6 (7.6–24.1)	95 (83–96)	98 (95–98)
Thioglycollate	128	413	12.6	18.9	13.4 (7.3–23.5)	19.4 (11.1–26.3)	98 (91–99)	99 (98–99)
C. parvum, pyridine extract	145	171	7.2	7.7	8.2 (6.8–8.2)	10.5 (8.3–10.5)	60 (42–76)	95 (92–95)
$NaIO_4$	144	143	9.6	12.2	3.0 (3.0–12.8)	10.0 (9.6–12.8)	32 (32–61)	98 (92–98)
Pyran copolymer	98	133	3.7	7.5	1.9 (0.2–2.2)	15.6 (9.1–15.6)	15 (4–15)	92 (81–92)
C. parvum, whole cells§	110	125	2.3	11.8	2.5	22.0	11	86
BCG‖	73	101	2.2	5.8	<0.2 (0–3.8)	12.8 (1.3–12.8)	2 (0–8)	89 (7–91)

* Macrophages were cultured for 2 or 48 h in DME-10% FBS before radiolabeling as described in Materials and Methods, and secreted ApoE was determined by densitometry of fluorograms. Intracellular ApoE was determined by the percentage of cells showing positive intracellular immunofluorescence. Data from a typical experiment are shown.

‡ Values in parentheses show the range observed in four to seven experiments for secreted ApoE and two to four experiments for immunofluorescence. For reasons not fully understood, ApoE secretion by resident macrophages from different batches of mice (possibly due to pathogens) varied from 7 to 26% of total secreted proteins. When secretion was at the lower part of the range, the secretion rates of the elicited macrophages were correspondingly low, and when secretion by resident macrophages was high, the ApoE secretion by the elicited macrophages was at the high end of the range.

§ These data are from a different experiment in which thioglycollate-elicited macrophages' ApoE was 22% of total secreted protein at 2 h, and 26% at 48 h.

‖ BCG-activated macrophages were variable in their time of onset of ApoE synthesis and secretion with time in culture. In some experiments (e.g., Fig. 2) little ApoE was detectable at 48 h, but ApoE secretion was always seen by 120 h in culture.

culture, however, the pyran-elicited macrophages contained intracellular ApoE, in parallel with the onset of secretion of ApoE.

We were able to localize ApoE intracellularly in actively secreting macrophages. As shown in Fig. 5, the thioglycollate-elicited and resident peritoneal macrophages that secreted biochemically measurable amounts of ApoE had a bright inclusion containing immunofluorescent materials. This immunofluorescence was located in the perinuclear area of macrophages corresponding to the Golgi complex (Fig. 6). In the resident and thioglycollate-elicited populations, virtually every macrophage was positive for ApoE antigen (Table II), whereas the occasional fibroblasts were negative. Nonpermeable cells did not stain for ApoE, suggesting that there is little, if any, ApoE on the surface of macrophages. In contrast, in the pyran-, whole *C. parvum*-, and BCG-elicited macrophages, few of the cells showed the punctate area of immunofluorescence (Fig. 5, Table II). Macrophages elicited with $NaIO_4$ or the pyridine extract of *C. parvum* secreted ApoE at intermediate rates, and mixed populations of macrophages negative and positive for ApoE by immunofluorescence were seen (Figs. 2 and 5, Table II). After 48 h in culture, when the $NaIO_4$-, BCG-, and pyran-elicited cells had been induced to secrete ApoE (Fig. 2), the percentage of cells containing ApoE increased to >89% (Table II). Thus, at this time the proportion of cells in the population judged to be secreting ApoE by its intracellular presence paralleled the measured secretion of ApoE. As macrophages were cultured for longer periods of time, ApoE secretion increased markedly (Fig. 3), and the size and extent of the immunofluorescent structures containing ApoE also increased (Fig.

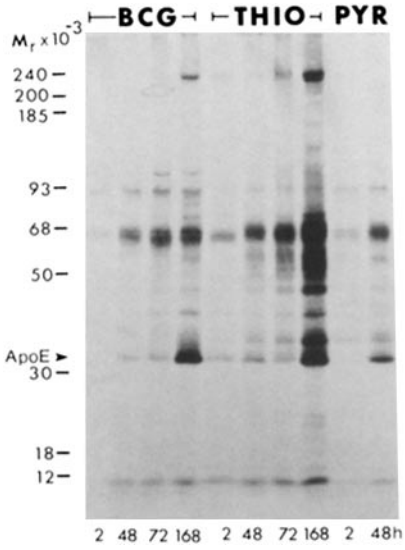


FIGURE 3. Secretion of ApoE and other proteins by macrophages during prolonged culture. Secreted proteins present in the conditioned medium from 2.5×10^5 radiolabeled macrophages are shown. Time in culture before labeling for 2 h with [^{35}S]methionine is indicated at the bottom. Lanes are from BCG-activated (BCG) or thioglycollate-elicited (THIO) macrophages cultured for 2, 48, 72, or 168 h or from pyran copolymer-activated (PYR) macrophages cultured for 2 or 48 h. Note the 5–10-fold increases in protein secretion per cell by thioglycollate- and BCG-elicited macrophages between 2 and 168 h in culture. M_r ($\times 10^{-3}$) markers and ApoE migration are indicated. Fluorographs were exposed for 3 d.

6).

Recruitment of New Macrophages into an Inflammatory Site Is Not Required for Suppression of ApoE Secretion. During response to chemical and immunologic stimuli, monocytes are recruited into the inflammatory site. To determine whether the suppression of ApoE secretion by immunologically activated macrophages was due to the recruitment of a population of immature macrophages that had not yet initiated ApoE synthesis, we studied ApoE secretion by peritoneal macrophages exposed in vivo to inflammatory stimuli in the absence of an influx of monocytes. As shown in Fig. 7, neither dexamethasone nor x radiation (2.5–5.0 Gy) affected ApoE secretion by thioglycollate-elicited or pyran-activated macrophages. Other biochemical properties of these inflammatory macrophage populations, including 5′-nucleotidase and elastase secretion, were similar for the untreated, dexamethasone-treated, or x-irradiated macrophages, as shown in Table III for resident and thioglycollate-elicited macrophages. Only the proliferative capacity of the newly recruited inflammatory macrophages failed to be induced in dexamethasone-treated animals.

Suppression of ApoE Correlates with Activation of Macrophages. The activation of macrophages is a multistep process that includes alterations in the capacity to secrete proteinases, to produce H_2O_2 in response to triggering stimuli, and to kill microorganisms and tumor cells (1–3). Accordingly, we compared the regulation of ApoE secretion with other phenotypic markers of macrophage activation.

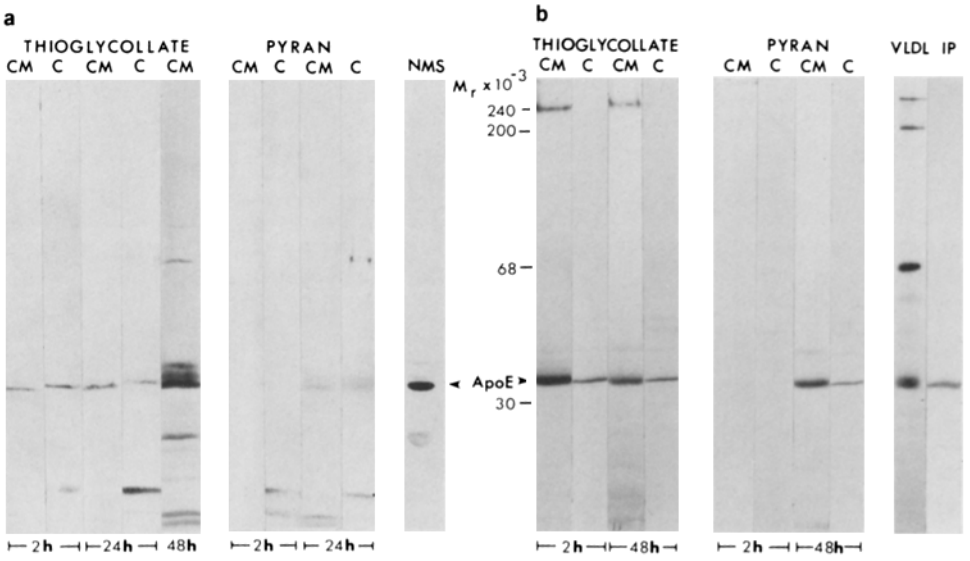


FIGURE 4. Immunoreactive ApoE present intracellularly (C) and in the conditioned medium (CM) of thioglycollate-elicited and pyran copolymer-activated macrophages in culture. (*a*) Immunoblot analysis of ApoE present in lysates of 2×10^6 macrophages at 2 and 24 h in culture, and CM collected from 2×10^6 cells at 2 h, 24 h, or 0–48 h in culture. ApoE (~0.3 μg present in 5 μl of normal mouse serum [NMS]) is shown at right. The bands migrating near the front of the nitrocellulose filter in both ApoE-containing and ApoE-deficient cells and CM are due to nonspecific binding of horseradish peroxidase conjugates to basic proteins. Note the glycosylated forms of ApoE in the 48-h thioglycollate CM and the degradation products present as fragments and aggregates. (*b*) Immunoprecipitation of ApoE from cell lysates and CM of 2.5×10^5 macrophages labeled for 2 h in [^{35}S]methionine after 2 or 48 h in culture. Total ^{125}I-labeled proteins in mouse VLDL and ApoE immunoprecipitated from VLDL (IP) are shown at right. The M_r 240,000 band present in CM lanes of thioglycollate-elicited macrophages is fibronectin that bound directly to *S. aureus*.

All macrophages with little or no ApoE secretion had enhanced rates of H_2O_2 production (Table IV). When BCG-, *C. parvum*-, or pyran-elicited macrophages were cultured for 48–72 h, they regained the ability to secrete ApoE (Table II, Fig. 3), whereas their rates of secretion of H_2O_2 decreased to rates comparable to the rates for resident and thioglycollate-elicited macrophages. When BCG-elicited macrophages were co-cultivated with peritoneal lymphocytes from animals challenged with killed BCG 4 d before lavage for 72 h, high H_2O_2 secretion was retained at 72 h and ApoE secretion remained barely detectable.

Enhanced secretion of plasminogen activator occurred both in all macrophages with decreased rates of ApoE secretion (BCG and pyran copolymer activated) and in macrophages with high rates of ApoE secretion (concanavalin A and thioglycollate elicited) (Table V). Metal-dependent elastase secretion was low in BCG-, pristane-, endotoxin-, and pyran copolymer-elicited macrophages secreting little or no ApoE, but was also low in resident macrophages that secreted abundant ApoE. 5′-Nucleotidase was high in resident macrophages but decreased in all inflammatory macrophages. Thus, plasminogen activator, elastase, β-glucuronidase, and 5′-nucleotidase were not useful for predicting ApoE secretion.

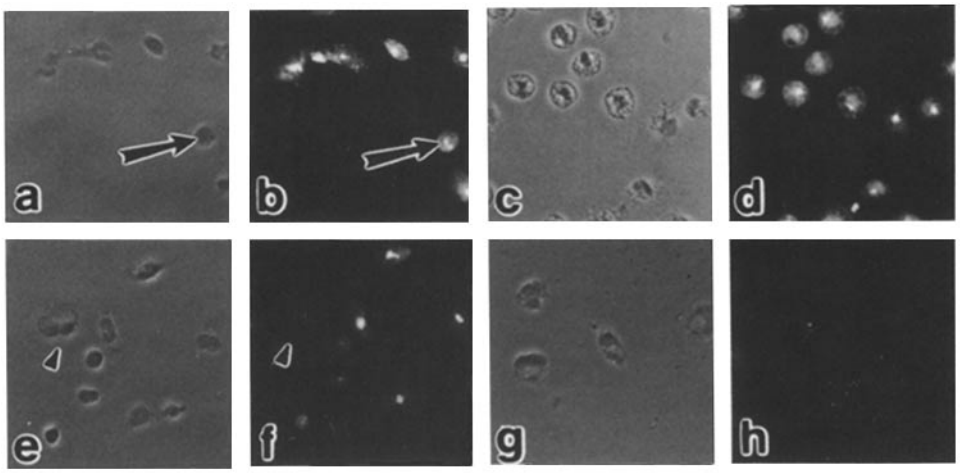


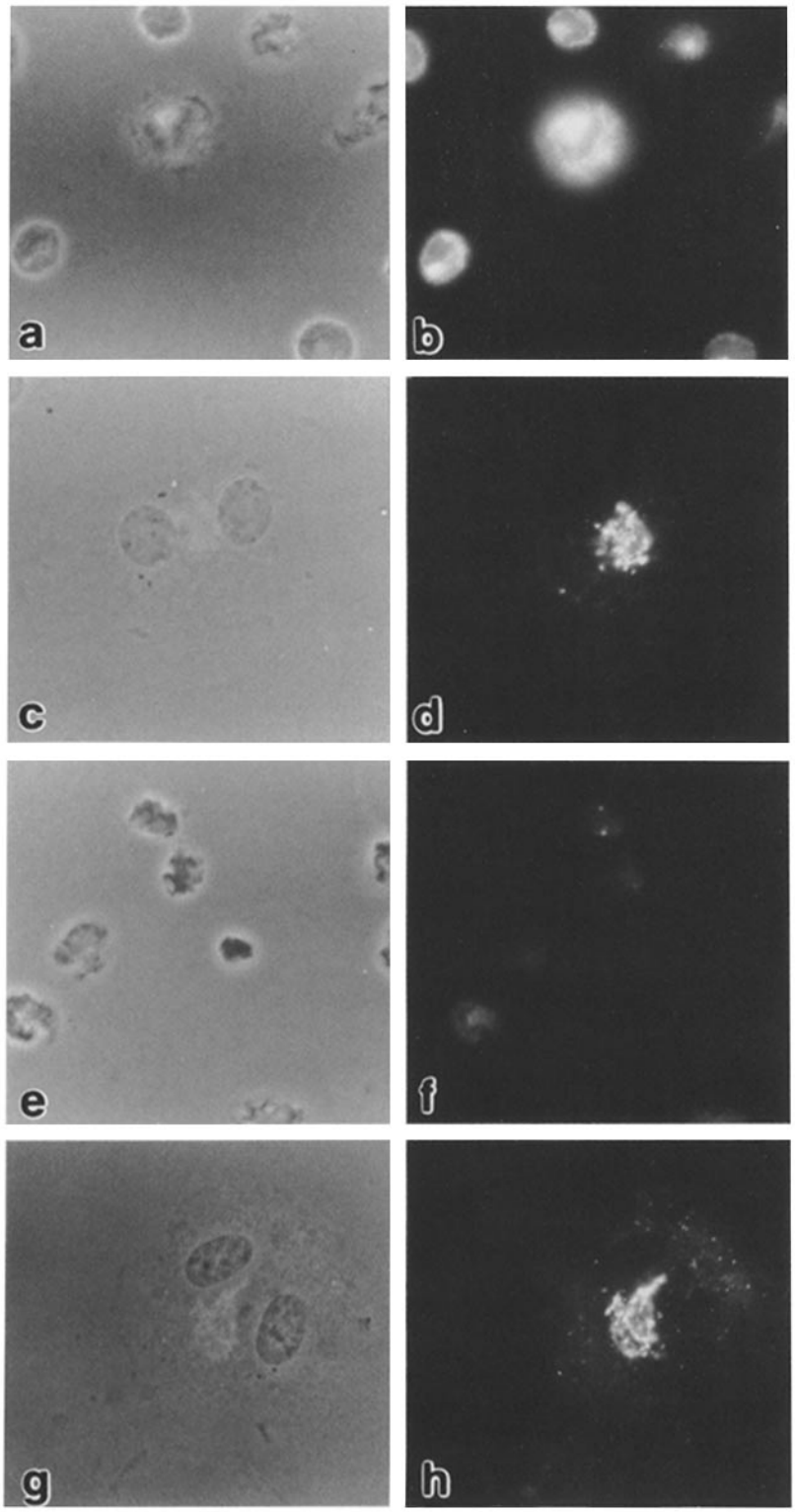
FIGURE 5. Immunofluorescent localization of ApoE in mouse peritoneal macrophages cultured for 2 h. (*a*, *b*) Resident macrophages; (*c*, *d*) thioglycollate-elicited macrophages; (*e*, *f*) $NaIO_4$-elicited macrophages; (*g*, *h*) pyran-elicited macrophages. In (*a*, *b*) arrows indicate a macrophage positive for ApoE immunofluorescence, and in (*e*, *f*) arrowheads indicate a negative macrophage. (*a*, *c*, *e*, *g*) Phase-contrast microscopy; (*b*, *d*, *f*, *h*) fluorescence microscopy. × 300.

BCG-activated macrophages from C3H/HeJ mice display most of the phenotype of activated macrophages but are defective in the final steps of activation, including production of cytolytic proteinase and binding of tumor targets, unless incubated with lymphokines (50). As shown in Fig. 8, on a per cell basis, BCG-activated macrophages from C3H/HeJ mice secreted little ApoE compared with resident macrophages, consonant with the secretion phenotype of macrophages from normal mice (Figs. 2 and 3) that do not have defective tumoricidal capacity. Injection of pyran copolymer into C3H/HeJ mice yielded macrophages that did not secrete detectable ApoE at 2 h in culture (not shown); however, the C3H/HeJ macrophages did not suppress ApoE secretion in response to endotoxin, in keeping with their genetic defect in endotoxin responsiveness.

Discussion

In the present study we have shown that the synthesis and secretion of ApoE are modulated by the functional state of macrophages. ApoE secretion was suppressed by treatment of macrophages in vivo with agents known to activate the cells for antimicrobial and antitumor functions, and ApoE synthesis was induced as the macrophages lost their activated state. Macrophages elicited with the sterile inflammatory agents thioglycollate broth and concanavalin A resembled the activated macrophages from BCG-, whole *C. parvum*-, endotoxin-, and pyran copolymer-injected mice in a number of respects, including low ecto-5′-

FIGURE 6. Changes in intracellular immunofluorescent ApoE during time in culture. Resident macrophages cultured for 2 h (*a*, *b*) or 168 h (*c*, *d*); BCG-elicited macrophages cultured for 2 h (*e*, *f*) or 168 h (*g*, *h*). (*a*, *c*, *e*, *g*) Double exposure of phase-contrast and fluorescence microscopy; (*b*, *d*, *f*, *h*) fluorescence microscopy. Note that in (*a*, *b*) ApoE is in a small, dense area, whereas in (*c*, *d*, *g*, *h*) the ApoE-stained area is much more extensive. In addition to Golgi region staining, some small peripheral vesicles are also positive in *h*. × 1000.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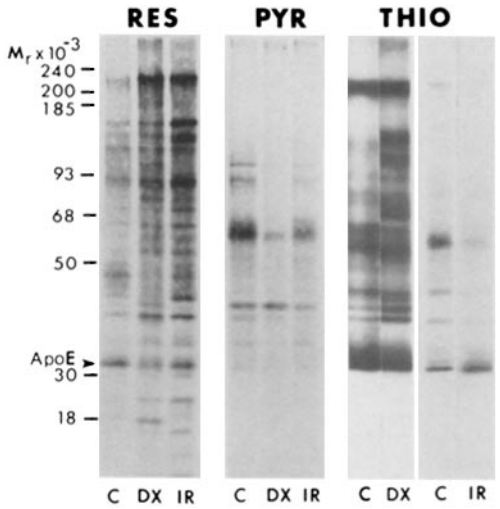

FIGURE 7. Secretion of [^{35}S]methionine-labeled proteins by peritoneal macrophages in dexamethasone-treated or x-irradiated mice. Acid-precipitable proteins are shown from the conditioned medium at 2 h in culture of 2.5×10^5 resident (RES), pyran copolymer-elicited (PYR), or thioglycollate-elicited (THIO) macrophages obtained from untreated control (C), dexamethasone-treated (DX), or x-irradiated (IR) mice.

TABLE III

Effects of Dexamethasone and x Irradiation on the Functions of Resident and Thioglycollate-Elicited Macrophages

Stimulus	Mouse treatment*	Macrophages recovered per mouse ($\times 10^6$)	5′-Nucleotidase	Elastase secreted (48 h, per 10^6 cells)	Plasminogen activator secreted (48 h, per 10^6 cells)	Relative ApoE secretion rate	Relative proliferation in L-cell-conditioned medium‡
			nmol/min/mg	*U*	*U*		
None	None	2.3 ± 0.4	74	2.2	2.6	1.0§	None
	Dexamethasone	1.4 ± 1.1	85	1.4	5.0	1.0	None
	x rays, 2.5 Gy	2.2 ± 0.5	98	2.0	5.0	0.9	ND‖
Thioglycollate	None	6.6 ± 2.5	3.8	15.6	75.8	1.8	High
	Dexamethasone	2.6 ± 1.4	7.2	15.6	38.2	2.0	None
	x rays, 2.5 Gy	2.0 ± 0.5	3.8	17.2	78.0	2.0	ND

* Treatments were as described in Materials and Methods.
‡ Clonigenic assays were performed as described in Materials and Methods.
§ ApoE secretion rate at 2 h in culture was determined from fluorograms of secreted proteins labeled with [^{35}S]methionine for 2 h and was normalized for cell protein. Secretion by untreated resident macrophages was set as 1.0.
‖ Not determined.

nucleotidase activity and increased secretion of plasminogen activator. However, the two populations differed in total protein secretion and in secretion of ApoE, elastase, and β-glucuronidase. Both types of elicited macrophages differed from resident macrophages in secretion of 5′-nucleotidase and plasminogen activator.

TABLE IV

Comparison of ApoE Secretion and H_2O_2 Production

Stimulus	ApoE secreted after 2 h in culture* (2 h, per 10^6 cells)	H_2O_2 secretion rate‡ (per 10^6 cells) after culture for:	
		2 h	72 h
	dpm × 10^{-3}	*nmol/min*	
None	4.6	< 0.04	< 0.04
Thioglycollate	14.4	0.11	0.10
C. parvum, pyridine extract	4.2	0.20	0.20
Endotoxin	1.2	0.58	0.12
Pyran copolymer	0.6	0.74	0.15
BCG	0.1	0.96	0.09
C. parvum, whole cells	0	1.02	0.18
BCG, boosted	0	1.28	1.12

* See Table I for relative ApoE secretion at 48 h.

‡ H_2O_2 secretion was triggered by 12-*O*-tetradecanoylphorbol-13-acetate.

TABLE V

*Cellular and Secreted Proteins of Mouse Macrophages Elicited by Various Inflammatory Stimuli**

Stimulus	Relative ApoE secretion rate‡	Secreted plasminogen activator (48 h, per 10^6 cells)	5′-Nucleotidase (per 10^6 cells)	Secreted elastase (48 h, per 10^6 cells)	β-Glucuronidase (per 10^6 cells)		Macrophages recovered per mouse (× 10^{-6})
					Cellular	Secreted at 48 h	
		U	*nmol/min*	*U*	*nmol/min*		
None	1.0	18	10.1	6.2	45	23	2.1
Concanavalin A, 750 μg	1.5	155	0.4	23.4	256	144	7.4
Thioglycollate							
0.2 ml	2.0	ND§	0.4	26.6	98	92	3.2
1.0 ml	2.0	121	0.5	27.2	114	92	8.1
2.0 ml	1.5	54	1.1	27.2	200	148	20.9
C. parvum, pyridine extract, 1 mg	1.0	24	0.4	8.8	34	78	3.3
$NaIO_4$, 0.5 ml of 5 mM	0.5	87	5.7	28.3	155	75	6.6
Complete Freund's adjuvant, 0.1 ml	0.4	24	1.0	9.8	44	31	5.6
Pristane, 0.1 ml	0.2	N.D.	0.5	5.0	34	27	3.1
Pyran copolymer, 25 mg/kg	>0.1	116	0.3	1.8	39	38	6.8
Endotoxin, 10 μg	>0.1	108	0.1	1.6	120	93	5.8
BCG, 5 × 10^6 organisms	>0.1	136	0.1	6.8	52	20	4.8
C. parvum, whole, 1 mg	>0.1	53	>0.5	4.7	N.D.	N.D.	4.9

* Data represent the means of two to eight experiments, each with duplicate or triplicate determinations.

‡ ApoE secretion, as determined from gels of labeled secreted proteins after 2 h in culture, was normalized for cell protein and resident macrophage secretion was rated 1.0.

§ Not determined.

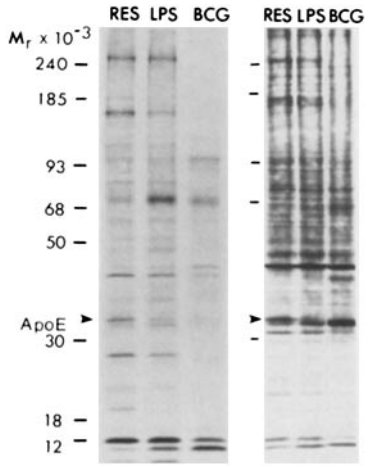

FIGURE 8. Secretion of [^{35}S]methionine-labeled proteins by peritoneal macrophages from C3H/HeJ mice after 2 h in culture. Acid-precipitable proteins are shown from the conditioned medium of 5×10^5 resident (RES), endotoxin-elicited (LPS), or BCG-elicited (BCG) macrophages.

Basu et al. (17, 18) previously reported that the uptake of materials containing cholesterol increased ApoE secretion. It is notable that the ApoE-secreting inflammatory macrophages elicited by thioglycollate, concanavalin A, and the pyridine extract of *C. parvum* all contained intracellular material. On the other hand, macrophages from whole *C. parvum*- and BCG-injected animals also had intracellular bacteria, but did not secrete ApoE. The intracellular storage of materials has been shown to influence microbicidal activity of macrophages (51, 52); however, the relationships between intracellular storage, macrophage activation, and ApoE secretion have not been fully established.

Although macrophage populations elicited either by nonspecific inflammatory agents or by activating agents in vivo contain macrophages newly recruited from blood and bone marrow (1–3, 33), these young cells were not the sole target for alteration of macrophage secretory phenotype by the inflammatory agents. The distinct patterns of protein secretion characteristic of the elicited populations were, in general, reproduced in the peritoneal macrophages exposed directly. Altered secretion of ApoE and elastase was induced in the peritoneal macrophages by thioglycollate broth or pyran copolymer under conditions in which the influx of new cells was inhibited. Although these phenotypically "stimulated" resident macrophages from irradiated or steroid-treated mice resembled macrophages from elicited populations in most respects, including protein secretion, they did not acquire the ability to proliferate in response to colony-stimulating factor, a property of immature macrophages.

In the series of steps associated with acquisition of the activation phenotype (1–3, 9, 11, 12, 48, 50), the step that correlates with the suppression of ApoE synthesis and secretion was the same as that associated with induction of H_2O_2 secretion. Induction of the capacity to secrete H_2O_2 correlated with loss of ApoE secretion by inflammatory macrophages. Macrophages capable of high rates of H_2O_2 secretion, including those elicited by BCG, whole *C. parvum*, pyran copol-

ymer, and endotoxin, had suppressed rates of ApoE secretion. As ApoE secretion was induced in these macrophages during culture, capacity for H_2O_2 secretion was lost. It is interesting to note that 12-*O*-tetradecanoylphorbol-13-acetate, the tumor promoter used to trigger H_2O_2 secretion, had no effect on ApoE secretion (not shown), in keeping with the observation that it is not a macrophage activator (53). However, ApoE secretion was not directly correlated with the ability to kill tumor cells via the cytolytic proteinase mechanism, because BCG-activated macrophages from C3H/HeJ mice that are defective in cytolytic proteinase secretion (50) showed suppression of ApoE secretion in a way resembling that of BCG-activated macrophages from normal mice.

The suppression of ApoE synthesis and secretion was not the only change in protein synthesis that occurred during macrophage activation. Overall protein secretion was decreased, and, with a number of stimuli, fibronectin and several other major secreted proteins were also suppressed, whereas cellular protein synthesis changed relatively little. Because there are many cellular and biochemical components to the activating inflammatory response in vivo (1–3), e.g., activation of the complement system, it is not surprising that pleiotypic effects are seen.

The mechanism by which activating substances suppress ApoE synthesis in macrophages is not known, but ApoE secretion is also regulated by substances that activate macrophages directly. Substances like endotoxin induce cytolytic activity and reactive oxygen production by macrophages in culture (54). In other work (55) we have shown that <1 ng/ml of endotoxin rapidly and specifically suppresses ApoE secretion when added to macrophages in culture. Although total RNA synthesis is decreased in cytolytically activated macrophages (56), the activating stimuli affected overall protein synthesis relatively little when the decreased size of the activated cells is taken into consideration. Thus, it is possible that mRNA species with short half-lives may be affected selectively. Preliminary experiments with RNA inhibitors support this hypothesis and favor transcriptional control of ApoE expression in macrophages; our data indicate that activated and nonactivated macrophages contain similar amounts of total translatable mRNA, but pyran copolymer-activated macrophages contain no detectable translatable mRNA for ApoE, whereas ApoE mRNA is a major translatable species (~2%) in thioglycollate-elicited macrophages (R. Takemura, S. Frisch, and Z. Werb, unpublished observations).

The proportion of macrophages that contained intracellular ApoE, as shown by immunofluorescence, paralleled the appearance of ApoE in the secretions of these cells. The secretion phenotype of macrophages changed dramatically during culture. In resident, nonspecific inflammatory, and activated macrophages, total synthesis and secretion of ApoE and other proteins also changed markedly after as little as 24 h in culture; by 24–96 h virtually all the macrophages became positive for ApoE immunofluorescence, and then, as the secretion of ApoE increased, the immunofluorescence of individual cells became more intense. The nature of the intracellular ApoE is not fully resolved. Macrophages that expressed this protein contained an intracellular pool equivalent to the amount secreted in a 2–4 h period. In contrast to the diffuse immunofluorescent intracellular staining seen in macrophages stained with antibodies to fibronectin

(19), ApoE was concentrated in the perinuclear area. As observed by ultrastructural immunocytochemistry, most of this ApoE is localized in Golgi cisternae (D. F. Bainton and Z. Werb, unpublished observation). Although the appearance of intracellular ApoE always paralleled ApoE secretion, further experiments are necessary to determine whether the cell-associated pool arises directly during synthesis as part of the secretory pathway or as a kinetically independent pool, or indirectly by an endocytic pathway.

Macrophages play a major role in lipoprotein metabolism. They are able to take up triglycerides, and cholesterol and cholesteryl esters from their environment via specific receptors on their surface for altered lipoproteins and chylomicron remnants (57–59). Within the cell, cholesteryl esters are hydrolyzed by lysosomal cholesteryl esterases (59, 60) and resynthesized within the cytoplasm (57, 60). Macrophages play an active role in the secretion of lipoprotein lipase (61), an enzyme involved in the regulation of lipoprotein metabolism. In vivo, macrophages are a prominent feature of the atherosclerotic plaque, where they appear as foam cells (62), and they are prominent in other areas of lipid deposition, including xanthomas. Although macrophages may contribute to ApoE or lipoproteins, it is now clear that ApoE secretion is not coupled to cholesterol excretion by macrophages (63). Whether the cells are performing a catabolic and debridement function in these situations, or whether there is a derangement in the normal metabolic function of the macrophage that leads to the alteration of the homeostatic means, has yet to be determined.

ApoE is a major protein found in the circulation as a component of the high density lipoprotein subclass C (HDL_C), on chylomicron remnants, and on β-migrating VLDL (25, 64, 65). ApoE is essential for the recognition and clearance of these lipoproteins by the receptors that recognize both apoproteins E and B (25, 65, 66). In the metabolic disorder, dyslipoproteinemia, there is a mutant form of ApoE that differs from the normal by a single amino acid substitution that is poorly recognized by hepatic lipoprotein receptors, and chylomicron remnants (β-VLDL) accumulate in the circulation with an accompanying hyperlipidemia (57). The contribution of macrophages to plasma ApoE is not known. However, ApoE synthesis in culture (55) may be increased in response to uptake of lipoproteins containing cholesterol (17, 18), and we have shown that macrophages in vivo and in culture regulate their expression of ApoE. The relative significance of the activation-mediated suppression of ApoE secretion and the enhanced ApoE secretion after cholesterol loading (17), or after prolonged time in culture for particular situations in vivo, remains unresolved.

Substances known to activate macrophages in vivo have profound effects on the lipid metabolism. Triglycerides are increased markedly in the circulation in humans with gram-negative infections, and there is an associated accumulation of β-VLDL (67). Similarly, rabbits infected with these organisms or injected with endotoxins also show a hypertriglyceridemia (68–70), consistent with the derangement of the metabolism of lipoproteins containing ApoE. Gram-negative organisms, endotoxins, and homopolymers such as pyran copolymer are activators of macrophages (1, 30, 71), and, as shown in this investigation, when injected into animals these agents decrease synthesis and secretion of ApoE by macrophages.

In addition to the transport and cellular recognition of lipids, ApoE in the absence of lipids may be important in the immune response. Lymphocytes have receptors for lipid-free ApoE (26, 72) that mediate regulation of their functions. ApoE itself (27) and some species of lipoproteins containing ApoE (26) suppress mitogen-induced B and T cell functions. Phospholipid turnover (27), IgG synthesis, T suppressor functions, and T helper functions (73) are all regulated by these lipoproteins, at concentrations seen in the supernatant fluid of macrophages in culture. Thus, ApoE may be one of the soluble mediators of T and B cell functions produced by macrophages, and the decreased secretion of ApoE by macrophages during their immunologic activation by macrophages, microorganisms, or lymphokines may promote the immune response.

Our data suggest that macrophages may play an important homeostatic role in lipid metabolism and in the immune response through the regulated synthesis of ApoE in lipoproteins. Further studies using intracellular ApoE as a probe for the state of activation of individual macrophages may provide important information on functional subpopulations of macrophages in inflammatory sites in vivo.

Summary

Macrophages are active secretory cells that display functionally distinct phenotypes that are regulated by inflammation. We have found that apoprotein E (ApoE), a component of plasma lipoproteins, was synthesized and secreted by resident and nonspecifically stimulated macrophages elicited with thioglycollate broth, but not by activated macrophages obtained from mice treated with bacillus Calmette-Guerin, pyran copolymer, whole *Corynebacterium parvum*, or bacterial endotoxin. ApoE represented ~1% of the newly synthesized protein and ~10% of secreted protein of resident and thioglycollate-elicited macrophages. ApoE from thioglycollate-elicited macrophages was indistinguishable from ApoE in mouse plasma lipoproteins, as determined by immunoreactivity, peptide mapping, and molecular weight. When specific antibodies were used to localize cell-associated ApoE, strong immunofluorescence was seen in the Golgi region of resident and thioglycollate-elicited macrophages immediately after removal from the peritoneal cavity, as well as after culture for up to 7 d. In contrast, activated macrophages did not synthesize or secrete ApoE to an appreciable extent and had no immunocytochemically detectable intracellular ApoE. When activated macrophages were cultured in medium containing serum, their activated state, as judged by production of H_2O_2, declined within 48–72 h in parallel with the induction of synthesis and secretion of ApoE and detection of intracellular ApoE by immunofluorescence. During prolonged culture the rate of synthesis and secretion of ApoE increased in both resident and activated macrophages. Therefore, the synthesis and secretion of ApoE may serve as markers for the functional state of macrophages.

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