lities.[7,16,17] The nonspecific adherence of cells at high concentra- carbonyl iron can reduce the number of cells recovered after nt to as low as 50% of the original spleen cell suspension.[9] Hence, l for using the minimal amount of carbonyl iron which will cause a m degree of macrophage depletion. Cell aggregation can also be ed by using an increased concentration of FBS (e.g., 15–20%) in ıe culture medium, applying more agitation during the incubation o keep the cells in motion, or decreasing the cell density in the from 1×10^7/ml to 1×10^6/ml.

oval of macrophages from a heterogeneous cell population by the arbonyl iron appears to be quite efficient when compared to other l depletion methods such as adherence to Petri dishes and composed of glass beads or Sephadex G-10, which may result in specific removal of other types of cells. The carbonyl iron tech- lso has the advantages of being highly reproducible in terms of y of macrophage-depleted cells and extent of depletion. Thus, it is ended for routine use where a reproducible and rapid fractiona- cells is important. It should be stressed that no one technique the total removal of macrophages from a mixed cell population. ı contamination of 1% or less of macrophages is critical (e.g., in uation of accessory cell functions), at least two depletion proce- ıould be used, preferentially one based on the phagocytic and the ı the adherence properties of the macrophages.

ıvel method employing a fluorescence-activated cell sorter to re- acrophages which have ingested fluorescein-conjugated latex par- ıpears promising.[18] However, this method is time consuming and ee of macrophage depletion may be comparable to that obtained bonyl iron treatment. A better method may be the use of mono- ntibodies. With the development of monoclonal antibody against cell determinants, the ideal antimacrophage serum may become e as the most specific reagent against macrophages. Since there is evidence to suggest that macrophages may be heterogeneous in e (e.g., have different antigenic markers) and function,[19] the ınal antibody must be directed at an antigenic determinant shared

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by all macrophages for the complete removal of this cell type. Once such a reagent against macrophages becomes available, then the physical depletion of the cells through rosetting techniques, cellular immunoabsorbent columns, or complement-dependent lysis will be more effective than any of the methods available at the present time.

[31] Preparation and Use of Monoclonal Antimacrophage Antibodies

By MAY-KIN HO and TIMOTHY A. SPRINGER

Introduction

Macrophages are a diverse family of cells originating from pluripotent stem cells in the bone marrow. Outside the marrow, macrophages can take the form of blood monocytes, Kupffer cells, alveolar macrophages, peritoneal macrophages, Langerhans cells, and in almost every organ, as "fixed tissue macrophages." In addition to variation in anatomical localizations, macrophages can exhibit heterogeneity in function and state of differentiation. The advent of hybridoma technology has allowed the identification of over 40 macrophage antigens, some of which are useful for distinguishing macrophages from other cells whereas others are associated with distinct subsets of macrophages.

In this section, we have chosen a number of monoclonal antibodies (MAb) with relatively restricted specificities for macrophages and summarized their characteristics. Possible applications of these antibodies are also described.

Preparation of Monoclonal Anti-Macrophage Antibodies

Procedures for the production of monoclonal antibodies have already been described in detail elsewhere.[1] Most of the antibodies summarized here were prepared by using macrophages, monocytes, or macrophage cell lines as immunogens.[2–4]

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Certain antigens, such as Mac-1, and histocompatibility antigens, are more immunodominant. Hence, MAb to these antigens may be repeatedly isolated from different fusions. To allow the production of MAb to less immunodominant molecules, Springer depleted several previously defined antigens from macrophage glycoproteins by affinity chromatography and used the effluent as immunogen. Several MAb recognising previously unidentified antigens were produced by this "cascade" procedure.[5]

In general, hybrids are screened for their ability to bind macrophages or to inhibit certain macrophage functions. Binding of MAb to cells can be detected by a secondary antibody labeled by a radioisotope, fluorescent dye, or enzyme conjugate. To avoid reactivity with the target cells, the second-stage antibody should be prepared in the same specie as the target cells, or absorbed with immunoglobulins from the appropriate species. A more elegant approach to decrease cross-reactivity is to use MAb as the secondary antibody as well. Several MAb against mouse[6] or rat[7] immunoglobulin subclasses and kappa chains are now available through the American Type Culture Collection (ATCC). Hybrids selected by the primary screen can be further characterized by (1) their reactivity pattern on a large number of normal cells, leukemic cells, and cell lines; (2) immunoprecipitation of the antigens recognized; and (3) *in situ* staining of tissue sections. Immunoprecipitation studies are especially useful because they provide an early indication of whether the newly isolated MAb have the same specificity as existing antibodies.

Macrophage Antigens Defined by Monoclonal Antibodies

Mouse Antigens

Antigens identified by some monoclonal antimacrophage antibodies are listed in Table I. Mac-1, the first macrophage antigen defined by a MAb, M1/70, is a general marker for distinguishing macrophages from lymphocytes. It is found on peritoneal macrophages elicited by a variety of agents, blood monocytes, alveolar macrophages, and free-lying macrophages in spleen, lymph node, and thymus.[8–10] Lymphocytes, Kupffer cells, and dendritic cells are Mac-1$^-$.[10] Mac-1 is not entirely macrophage-specific because granulocytes, 50% of bone marrow cells and natural killer cells also bear this antigen.[11] 1.21J, an independently isolated MAb, also recognizes Mac-1.[12]

Several antigens are expressed on only subpopulations of macrophages. They include AcM.1,[13] 54-2[14,15] M43, M57, M102, and M143.[16] AcM.1 is only detected on activated macrophages induced by pyran and *Corynebacterium parvum*, but not on monocytes, resident macrophages, and macrophages elicited by thioglycolate, peptone, and mineral oil. The 54-2 antigen is strongly expressed on surfaces of thioglycolate-induced, but not resident peritoneal macrophages. Mac-2 was originally found to have a similar distribution by immunofluorescence and immunoprecipitation.[17] However, recent immunohistochemical studies show that 5% of resident macrophages are strongly Mac-2$^+$ in the cytoplasm.[10] The remaining 95% show much weaker, but definite staining. It seems that only cells with large amounts of Mac-2 internally express this antigen on their surfaces. In addition to peritoneal macrophages, *in situ* staining showed that Mac-2 is found on macrophages of all lymphoid and nonlymphoid tissues examined thus far. Interdigitating dendritic cells and Langerhans cells are also Mac-2$^+$.[10] Another antigen identified from the same fusion, Mac-3, is present in all Mac-2$^+$ cells.[18] In addition, megakaryocytes and endothelial cells of postcapillary venules are Mac-2$^-$ but Mac-3$^+$.[10]

For the identification of macrophages in cell suspensions, F4/80 can be used. The F4/80 antigen is present on macrophages from the peritoneal cavity, spleen, thymus, lymph node, and lung. Blood monocytes and macrophages derived from *in vitro* bone marrow cultures also express this antigen. Lymphocytes, granulocytes, and fibroblasts are negative.[19] It seems that the amount of F4/80 expressed increases with the maturity of macrophages.[20]

Human Antigens

A summary of antigens on human monocytes/macrophages is presented in Table II. These antigens can be roughly categorized into three

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[7] T. A. Springer, A. Bhattacharya, J. T. Cardoza, and F. Sanchez-Madrid, *Hybridoma* **1,** 257 (1982).
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TABLE I
RAT MONOCLONAL ANTIBODIES DEFINING MOUSE MACROPHAGE ANTIGENS

Antibody		Antigen		Distribution[a]				
Designation	Subclass	Designation	Polypeptide chains	Fluid cells	Solid tissue	Functional significance[a]	Lysis	References
M1/70[b]	IgG_{2b}	Mac-1	190,000 105,000	PEM, PM, M, 50% bone marrow, mature Mac lines	Mac in splenic red pulp, LN sinuses; alveolar Mac	Inhibits binding to Mac CR_3; blocks induction of Mac-3 expression on myeloblast; ADCC and NK effectors	Weak	8–11, 42–45
1-21J	NR	Mac-1	180,000 94,000	J774 Line	NR[c]	NR		2, 12
M3/31[b] M3/38[b]	IgM IgG_{2a}	Mac-2	32,000	TG-PEM, mature Mac lines	Mac in GC, red pulp, LN sinuses, and thymus; DC in red pulp PALS, and LN; alveolar Mac, Kupffer cells; Langerhans cells, epithelial cells	NR	NR	5, 10, 17
M3/84[b]	IgG_1	Mac-3	110,000	PEM, PM, Mac lines, some myeloid and B lymphoid lines	Same as for Mac-2, plus megakaryocytes and PCV endothelial cells	NR	NR	5, 10, 18

54-2[d]	IgG_{2a}	Mac-4	NR	BM-Mac, TG-PEM, mast cells	NR	NR	NR	14, 15
M3/37	NR	Mac-4	180,000	TG-PEM	NR	NR	NR	5
F4/80	IgG_{2b}	F4/80	160,000	PEM, PM, M, splenic Mac, thymic Mac, 8% BM, Mac lines, alveolar Mac	NR	Increases with maturity of Mac	–	19, 20
2.4G2	IgG	FcRII	47–70,000	Mac, B lymphocytes, Mac lines, PMN	NR	Inhibits binding to FcRII	NR	2
M43	IgM	M43	NR	30–40% PEM, BM-Mac	NR	Cytotoxic for lymphokine-activated Mac	+	16
M57	IgG_{2b}	M57	NR	20–30% PEM, BM-Mac, PMN	NR	Cytotoxic for ADCC effectors	+	16
M102	IgG_1	M102	NR	20–30% PEM, BM-Mac	NR	Cytotoxic for ADCC effectors	+	16
M143	IgG_{2a}	M143	NR	10–30% PEM, BM-Mac	NR	NR	+	16
AcM.1	IgG_{2c}	AcM.1		Pyran and *C. parvum*-PEM	NR	Cytotoxic for tumorcidal macrophages	+	13

[a] ADCC, Antibody-dependent cellular cytotoxicity; BM, bone marrow, CR_3, Type III complement receptor; DC, dendritic cells; GC, germinal center, LN, lymph node; M, monocytes; Mac, macrophages; NK, natural killer; PALS, periarteriolar lymphatic sheath; PCV, postcapillary venule; PEM, peritoneal exudate macrophages; PM, resident peritoneal macrophages; PMN, polymorphonuclear cells; TG, thioglycolate.

[b] Cell lines available through American Type Culture Collection (ATCC).

[c] NR, Not reported.

[d] 54-2 and M3-37 precipitate polypeptides which coelectrophorese (M. K. Ho, unpublished).

TABLE II

MONOCLONAL ANTIBODIES DEFINING HUMAN MONOCYTE ANTIGENS[a]

Antibody		Antigen					
Designation	Subclass	Designation	Polypeptide chains	Distribution[b]	Functional significance[b]	Lysis	References
OKM1	IgG_{2b}	OKM1	NR[c]	PMN, null cells, blood monocytes	NR	+	21
M1/70[d]	IgG_{2b}	Mac-1	NR	PMN, NK, and ADCC effectors, blood monocytes	Inhibits binding to CR_3 on PMN; blocks induction of Mac-3 expression on NK and ADCC effectors	NR	11, 42, 44
Anti-Mo-1	IgM, IgG_{2a}	Mo-1	155,000 94,000	Monocytes, PMN, null cells, PM, immature BM, HL-60, U937, and KG-1 cells. Mac and monocytes in splenic red pulp, LN sinusoids, histiocytes	Inhibits binding to CR_3 on monocytes	+	22, 32
Anti-Mo-2	IgM, IgG_{2b}	Mo-2	55,000	Monocytes, 7% BM, PM, HL-60, cultured monocytes, Mac and monocytes in splenic red pulp, LN sinusoid and GC	"Pan-monocyte antigen"	+	22, 32
Anti-Mo-3	IgM	Mo-3	NR	Cultured monocytes, 6% BM, HL-60, and U937 lines	NR	NR	25
Mac-120	NR	Mac-120	120,000	37% blood monocytes	Lymphokine production, accessory cells for T cell proliferation to Con A and antigens	+	26

63D3[d]	IgG_1	63D3	200,000	Weak on PMN monocytes	Accessory cells for mitogen-induced proliferation	NR	23, 46
MϕP-15	IgG_1	MϕP-15	NR	70% monocytes, 79% pleural Mac or PM	NR	NR	24
MϕP-9	IgG_{2b}	MϕP-9	NR	Most monocytes, pleural Mac and PM; 20–40% PMN	NR	NR	24
MϕS-1	IgG_{2a}	MϕS-1	NR		NR	NR	24
MϕS-39	IgG_{2a}	MϕS-39	NR		NR	NR	24
C10H5	IgM	C10H5	NR	Blood monocytes, <5% BM	Inhibits growth of myeloid/monocytic stem cell	+	27
D5D6	IgM	D5D6	NR	Blood monocytes, <5% BM, HL60		+	27
MMA	IgM	MMA	NR	Blood monocytes, PMN, U937, HL60, and some T cell lines	Accessory cells for T cell proliferation to mitogen and antigen, cytotoxic for precursors of granulopoietic colony forming cells	+	4
UC45	IgM	UC45	66,000	Monocytes, neurons	Specific for α chains of fibrin	NR	28, 29
10-75-3	IgG_{2a}	MPA	135,000	Blood monocytes, platelets, HL60	NR	+	30, 31
M206	IgG_{2a}	M206	180,000	Blood monocytes, U937, weak on PMN	Accessory cell for PWM-induced proliferation	+	3

[a] All antibodies listed, except M1/70, are mouse monoclonal antibodies.

[b] ADCC, Antibody-dependent cellular cytotoxicity; BM, bone marrow, Con A, concanavalin A; CR_3, Type 3 complement receptor with specificity for C3bi and its degradation products; GC, germinal center; LN, lymph node; Mac, macrophage; NK, natural killer; PMN, polymorphonuclear cells; PWM, pokeweed mitogen.

[c] NR, Not reported.

[d] Available through American Type Culture Collection.

roups. The first group comprises antigens found on blood monocytes/ nacrophages and polymorphonuclear cells, but not on lymphocytes. hey include OKM1,[21] Mac-1,[11] Mo-1,[22] 63D3,[23] MϕP-9, MϕS-1, MϕS-9,[24] and M206.[3] This group of markers serves to distinguish macrophages nd monocytes from cells of the erythroid and lymphoid lineage.

The second group, including Mo-2,[22] Mo-3,[25] Mac-120,[26] MϕP-15,[24] 10H5, D5D6,[27] UC45,[28,29] and 10-75-3,[30,31] are on all or a subset of nonocytes/macrophages, but not on lymphocytes or granulocytes. Therefore, they can be used to define monocytes from other hematopoietic cells.

The last group represents monocyte antigens which are expressed on nrelated cell types. For example, MMA[4] is found on abnormal T cells whereas UC45[28] is expressed on neurons.

Most of the data on distribution presented in Table II are from studies on cell suspensions. The only two antigens examined on tissue sections are Mo-1 and Mo-2.[32] Anti-Mo-1 stains polymorphonuclear cells and a ew large mononuclear cells in splenic red pulp and lymph node sinusoids. Mo-2 is not found on granulocytes, but on large mononuclear cells in the ed pulp, lymph node sinusoids, and germinal centers.

Applications

Identification of Macrophages in Vitro

Immunofluorescence. The most routine procedure for identifying macrophages is by indirect immunofluorescence. The cells to be assayed are

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incubated with saturating amounts of an antimacrophage MAb, washed, and incubated further with an excess of fluoresceinated second-step antibody. The secondary antibody should be specific for the primary Mab, but not cross-react with the target cells. Because the Fc receptors on macrophages can bind antibodies, several precautions should be taken: (1) $F(ab')_2$ fragments of the second stage antibody should be used; (2) avoid repeated freezing and thawing of the antibody reagents; (3) store antibodies at 4° after deaggregation by ultracentrifugation at 100,000 *g* for 1 hr; and (4) include a control MAb of the same isotype as the primary MAb, but with an irrelevant specificity.

Some antigens are modulated and possibly internalized after interaction with antibody, hence leading to erroneous results. This problem can be minimized by carrying out procedures at 4° and by including 10 m*M* sodium azide in the incubation and washing buffers.

The stained cells can be centrifuged onto glass slides, fixed, and examined under an UV microscope. Alternately, they can be analyzed by the fluorescence-activated cell sorter (FACS)[33] For this purpose, the stained cells should be filtered through nytex (Small Parts, Miami, Fl #CMN-37) to remove cell clumps. The anti-Mac-1 MAb has been found in some cases to cause agglutination of macrophages. This can be prevented by lowering the antibody concentration. In addition to the number of positive cells, FACS analysis can also provide information on the relative fluorescent intensity per cell. Details on this procedure are discussed in this volume [19].

Immunofluorescent staining has also been performed on adherent cell monolayers.[34] Briefly, macrophages are allowed to adhere onto glass cover slips. After fixation in 1% paraformaldehyde (to prevent antigen redistribution), the cells are stained by placing the cover slip, cell-side-down, onto 15 μl of antibodies.

Autoradiography. This procedure allows the visual localization of bound antibody on individual cells. Both cell suspensions or adherent cell monolayers can be used. The method of labeling is essentially the same as that for immunofluorescence except that the second antibody is radiolabeled. Labeled cells on glass slides or cover slips are dried, fixed, and coated with photographic emulsion.[19,35] After an empirically determined time, the slides are developed, and counterstained with Giemsa. When examined by light microscopy, the bound antibodies can be localized to areas with black silver grains. Both the morphology and relative quantity

[33] L. A. Herzenberg and Lenore A. Herzenberg, *in* "Handbook of Experimental Immunology" (D. M. Weir, ed.), 3rd ed., Chapter 22. Blackwell, Oxford, 1978.
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of antibody bound to cells can be determined simultaneously. However, this procedure is tedious and time consuming.

Rosetting. Cells sensitized with an antimacrophage MAb can be detected by binding to erythrocytes which have been coated with $F(ab')_2$ fragments of a secondary antibody by chromic chloride.[36,37] Usually, the sensitized cells and erythrocytes are mixed in a ratio of 1:40 or 50, centrifuged at 200 *g* for 5 min, and incubated for 1–5 hr at 4°. The pellet is then gently dispersed and cells stained by acridine orange or toluidine blue. The number of positive cells can be determined by counting the cells with five or more attached erythrocytes.

Enrichment or Depletion

Immunofluorescence. Fluorescent cells prepared as indicated above can be separated according to their fluorescence intensity on the FACS.[33] This method offers the advantage that almost pure populations of positive as well as negative cells can be recovered at relatively high yield. If only the positive (or negative) fraction is needed, the time of separation can be reduced by starting with cells that are enriched for this fraction by another procedure which gives less purity.

Complement-Mediated Lysis. There are both IgM and IgG MAb which can lyse macrophages in the presence of complement (see Tables I and II). Conditions leading to lysis are more stringent than those for binding. For each MAb used, a titration has to be performed to determine the optimal concentrations of MAb and complement. It is advisable to screen a number of complement batches from different animal sources to obtain the highest specific lysis but the lowest level of toxicity (lysis in the absence of MAb). Some MAb, such as M57 and M102, are indirectly cytotoxic.[16] They require the presence of a facilitating (second step) antibody to mediate lysis. Again, a MAb of the same isotype, but with an irrelevent specifity, should be included as control.

Depending on the antigen density, not all cells which show binding with a MAb will be lysed. Sometimes, several rounds of treatment are required to lyse most of the positive cells. Another approach is to use a mixture of MAb which recognize different antigenic determinants on the same molecule or cell.

Rosetting. Rosetted cells can be separated on Percoll[36] or Ficoll–Hypaque[37] density gradients. The bound erythrocytes can be dissociated from cells by either hypotonic lysis or by incubation of excess second-stage antibody followed by density gradient centrifugation. Relatively large numbers of cells (up to 10^9) can be separated by this procedure.

[36] E. P. Rieber, J. Lohmeyer, D. Schendel, and G. Riethmuller, *Hybridoma* **1**, 59 (1981).
[37] E. T. Dayton, B. Perussia, and G. Trinchieri, *J. Immunol.* **130**, 1120 (1983).

Localization of Macrophages in Situ

In addition to studies of cell suspensions, *in situ* staining of macrophages is important for the characterization of antimacrophage MAb because of the following reasons: (1) macrophages in tissue sections can be localized and examined in their "natural" state; (2) cell suspensions prepared from lymphoid or nonlymphoid organs may not be representative of the cellular composition of these tissue;[9,38] (3) immunohistochemistry allows the study of cells and structures, such as Kupffer cells and postcapillary venules, which are difficult to isolate.

Depending on the nature of the antigen, the tissue of interest can be frozen in OCT compound (Miles laboratories, Elkhart, IN) or fixed and embedded before sectioning. Optimal conditions for the localization of different antigens can vary greatly. Mac-1 is destroyed by fixation in alcohol, requiring study in frozen sections.[9] In contrast, Mac-3 and Ia antigens are readily detected in alcohol-fixed tissue embedded in polyester wax. Mac-2 is even more stable. It is routinely localized in tissue processed in Carnoy's fixative and embedded in paraffin.[10] Before staining, the embedding material has to be removed. Paraffin is soluble in xylene whereas OCT is water soluble.

Tissue sections are generally labeled by immunoperoxidase[39] or immunofluorescence. The former involves overlaying the sections sequentially with the chosen MAb, excess bridging antibody, and a peroxidase antiperoxidase (PAP) complex. In the case of rat MAb, rabbit anti-rat Ig and rat PAP have been used. Endogenous peroxidase can be quenched by incubating with 0.3–0.6% H_2O_2 before staining. A modification of this technique employs a primary MAb, biotinylated secondary antibody, and a preformed avidin–biotinylated peroxidase complex (ABC).[40,41] The ABC system is more sensitive than the PAP technique. Furthermore, the antibody in the PAP complex has to be made in the same specie as the MAb, whereas an universal ABC can be used for MAb prepared in different species. Sometimes, staining can be increased by using four layers of antibodies. Peroxidase activity is detected by incubating with diaminobenzidine (DAB) and H_2O_2. To allow identification of cells and structures, the sections can be counterstained with hematoxylin or methyl green.

Immunohistochemical studies on Mac-1, Mac-2, and Mac-3 have allowed the classification of the macrophage-dendritic cell family into two

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[40] S. M. Hsu, L. Raine, and H. Fanger, *Histochemie* **29**, 577 (1981).
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groups. Free-lying, round macrophages are positive for all three antigens whereas cells with dendritic morphology (excluding follicular dendritic cells) are Mac-1$^-$, but Mac-2$^+$ and Mac-$^+$.[10]

Functional Studies

The functional significance of antigens can be studies by performing the biological assay of interest in the presence of the appropriate MAb or after depletion of cells bearing the corresponding antigen. Control MAb reacting against other cell surface structures should be included in the assay.

Functional assays were used to select for and study an antibody to macrophage Fc receptor.[2] The M1/70 MAb inhibits the binding of C3bi to type III complement receptors (CR_3) on mouse macrophages and human polymorphonuclear cells. Binding to CR_1 and FcR are unaffected.[42] Anti-Mo-1 also has the same activity on human monocytes.[32] Therefore, it appears that these antigens are associated with the function of or identical to the CR_3. Anti-Mac-1 also inhibits the expression of Mac-3 on a mouse myelomonocytic line, M1, and a human monoblast line, U937, during induction by various agents.[43,44] This suggests that Mac-1 plays a regulatory role in macrophage differentiation. In addition, M1/70 defines the cells in human peripheral blood and mouse peritoneal exudate which have natural-killing activity.[11,45]

Some of the other MAb examined for functional significance include Mac-120, 63D3,[46] MMA, M206, M43, C10H5, and D5D6. The first four MAb are associated with accessory cells required for mitogen- or antigen-induced lymphocyte proliferation. M43 is cytotoxic for killer macrophages activated by lymphokines. MMA is cytotoxic for precursors of granulopoietic colony-forming cells whereas C10H5 and D5D6 inhibit the growth of myeloid/monocyte stem cells.

Acknowledgments

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[32] Elimination of Macrophages with Silica and Asbestos

By ELLIOTT KAGAN and DAN-PAUL HARTMANN

Silica and asbestos are naturally occurring, silicon-containing compounds which are widely distributed within geologic deposits in the earth's crust. Mechanical comminution of these mineral species produces particles which exhibit a diverse spectrum of size distributions. Asbestos differs from silica in that, unlike silica, asbestos particulates have a fibrous character (i.e., a length : width aspect ratio of greater than 3 : 1).

Certain types of asbestos and silica are toxic to macrophages, both *in vitro* and *in vivo*.[1-3] When macrophages are exposed *in vitro* to these toxic agents, they exhibit extreme cytoplasmic vacuolation, karyopyknosis, disappearance of pseudopodia, and effacement of plasma membranes.[3] Similar morphologic abnormalities have been observed in alveolar macrophages after experimental silica or asbestos inhalation in animals.[4,5] The cytoplasmic enzyme, lactate dehydrogenase, has proved to be a useful marker for assessing macrophage cytotoxicity induced by these mineral dusts.[6] Thus, the release of this enzyme into macrophage culture supernatants provides confirmatory evidence of macrophage lysis. The lethal action of silica is believed to result from a hydrogen bonding interaction of silicic acid with membrane protein and phospholipid moieties.[6] Chrysotile asbestos, on the other hand, appears to exert its cytotoxic effect through an electrostatic interaction of its surface magnesium groups with ionized carboxyl groups of membrane glycoprotein sialic acid residues.[6]

Silica does not appear to be directly cytotoxic to either T or B cells *in vitro*.[7] One study has, however, suggested that some silica preparations may suppress lymphoproliferative responses to concanavalin A and lipopolysaccharide mitogens in a macrophage-independent fashion.[8] Chrysotile asbestos, on the other hand, does not affect the viability of lympho-

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