# Immunomicrospheres: Reagents for Cell Labeling and Separation

A. Rembaum and W. J. Dreyer

*Summary.* Immunomicrospheres are specially designed microscopic particles that have antibodies or similar molecules chemically bound to their surfaces. The antibody-coated microspheres react in a highly specific way with target cells, viruses, or other antigenic agents. Immunomicrospheres may be synthesized so that they incorporate compounds that are highly radioactive, intensely fluorescent, magnetic, electron opaque, highly colored, or pharmacologically active. These various types of microspheres may be coated with pure, highly specific monoclonal antibodies obtained by the new hybridoma cell cloning techniques or with conventional antibody preparations. Some of the many present and potential applications for these new reagents are (i) new types of radioimmune or immunofluorescent assays, (ii) improved fluorescence microscopy, (iii) separation of cells on the basis of the fluorescent, electrophoretic, or magnetic properties of bound immunomicrospheres, (iv) markers for use in several types of electron or standard light microscopy, and (v) delivery of lethal compounds to specific undesirable living cells. The combination of the various new types of synthetic microspheres and the newly available homogeneous antibodies offers new opportunities in research, diagnosis, and therapy.

Each antibody molecule has binding sites that react in a specific manner with particular molecular shapes. It is believed that an animal can make more than a million different kinds of antibodies, and hence the immune system provides a source of reagents that react specifically with almost any type of molecule or disease organism. Although the normal function of antibodies is to destroy foreign organisms or the animal's own cancer cells, or to neutralize toxic substances, use of their specificity has been extensive in both research and clinical assay systems. One example is in studies of the structure and properties of molecules that are exposed on the surfaces of the outer membranes of cells (*1*). Antigenic differences in cell surface molecules are also widely used for cell classification and separation. For these reasons, intensive efforts have been made to detect, characterize, and quantify these molecules. In recent years, antibodies that are chemically modified with special labels have been used in many applications. Antibodies are coupled to either radioactive isotopes, fluorescent dyes, biological macromolecules, or synthetic particles (*2*). These reagents have been applied successfully to the identification of many diverse types of cells, such as subpopulations of B and T lymphocytes (*3*,*4*) and neural and Schwann cells (*5*).

Another example in which the diversity and specificity of antibodies is exploited is the analysis of various soluble molecules such as proteins or hormones. Also, labeled antibodies or antigens have proved extremely valuable in radioimmune and immunofluorescent assays. A practical application of these immunological approaches is in diagnostic tests of a large number of diseases and, in some cases, in their therapy. Unfortunately, many of the previously used reagents suffer from either lack of stability, nonspecific interactions, or restricted dimensions of the labels. For example, $^{125}I$, commonly used in radioimmune assays, has a short half-life; ferritin, a well-known cell surface marker useful for electron microscopic studies (*6*), cannot be used to distinguish cell subpopulations under the light microscope; and fluorescence labeled antibodies are limited in their sensitivity and bleach rapidly under illumination.

We have expanded the range of labels that are available for use in conjunction with antibodies. The new reagents are specially designed polymeric microspheres that may be synthesized in such a way that they have built into them a wide variety of different molecular labels or useful substances. Such microspheres have been coated with many types of antibodies; but homogeneous, pure antibodies produced by means of the new hybrid cell cloning methods are particularly useful when combined with these microsphere reagents.

In this article, we discuss several different methods that have been used in the synthesis of microsphere reagents and then elaborate on the present and potential applications for the immunomicrospheres of various types.

## Requirements

An essential requirement of reagents for cell tagging is the absence of nonspecific interactions between the immunomicrosphere and cells or other substances to be analyzed. Nonspecific interactions occur generally with hydrophobic particles such as polystyrene latex or polymethylmethacrylate. A pronounced hydrophilic surface character is, therefore, one of the essential conditions for the successful application of immunomicrospheres.

The presence of functional groups constitutes an equally important requirement. Hydroxyl and carboxyl groups are generally used for binding proteins by the cyanogen bromide (*7*) or carbodiimide method (*8*), respectively. Aldehyde groups, on extended molecular chains, are particularly desirable since they seem to bind proteins to microspheres at a faster rate and with greater convenience than many other functional groups (*9*). It is desirable to obtain microspheres that are insoluble in a variety of solvents in order to allow derivatization reactions to proceed in different media and in order to carry out fixing, dehydration, and critical-point drying of cells for electron microscopy. Finally, microspheres must be stable and not aggregate in a physiological *p*H range and in media in which cells are maintained or cultured.

## Synthesis and Characterization

Most of the above requirements may be met by selecting the synthetic procedure and composition of initial materials for the synthesis. So far, three polymerization techniques have been successfully applied. (i) Emulsion polymerization is a classical and fairly well understood pro-

Dr. Rembaum is a technical group supervisor at the Jet Propulsion Laboratory, California Institute of Technology, Pasadena 91103. Dr. Dreyer is a professor in the Division of Biology, California Institute of Technology, Pasadena 91125.



0036-8075/80/0425-0365$01.25/0 

cess for the formation of lattices and colloids; (ii) polymerization by ionizing radiation is a more recent technique used to produce spherical polymeric particles; and (iii) aldol condensation-polymerization reaction of glutaraldehyde is a hitherto unexplored method for the formation of small particles with functional groups on the surface.

*Emulsion polymerization*. The polymerization is carried out in an aqueous medium with the following main components: methyl methacrylate (MMA), methacrylic acid (MA), and 2-hydroxyethyl methacrylate (HEMA) (*10*). The last-mentioned, HEMA, a monomer used in the manufacture of contact lenses, imparts hydrophilicity to the polymeric particles produced. Ethylene glycol dimethacrylate in small amounts is a cross-linking agent and ensures insolubility in organic solvents. Methacrylic acid provides reactive carboxyl functions and stability because of repulsive charge interactions of polar carboxyl groups. A free radical polymerization initiator (ammonium persulfate) and an emulsifier (sodium dodecyl sulfate) are also used.

The size of the spheres is dependent on the concentration of monomers used in the copolymerization reaction. The diameter of the spheres can be decreased from 340 to 35 nanometers by reducing the total monomer concentration from 35 to 3 percent. A linear relation between these parameters is observed (*9*).

The spheres are quite uniform in size with a standard deviation generally less than ±1.5 percent of their average diameter. A density of 1.23 ± 0.01 gram per centimeter has been measured for these copolymer methacrylate spheres by centrifugation on a continuous sucrose gradient.

Since polymerization is carried out in an aqueous emulsion system, a high concentration of hydrophilic hydroxyl and carboxyl groups is present on the surface of the spheres. At neutral and alkaline *p*H, the spheres are negatively charged as a result of the ionization of the carboxyl groups. At *p*H values below 5.5, when the extent of ionization of the carboxylic groups is decreased, the particles aggregate. Hydrogen ion titration measurements indicate that copolymer latex spheres with an average diameter of 60 nm contain approximately 4200 titratable carboxyl groups per sphere. The hydrophilic, negatively charged surface not only prevents the spheres from aggregating in the absence of an emulsifying agent but also prevents the spheres from binding nonspecifically to the negatively charged cell surfaces.

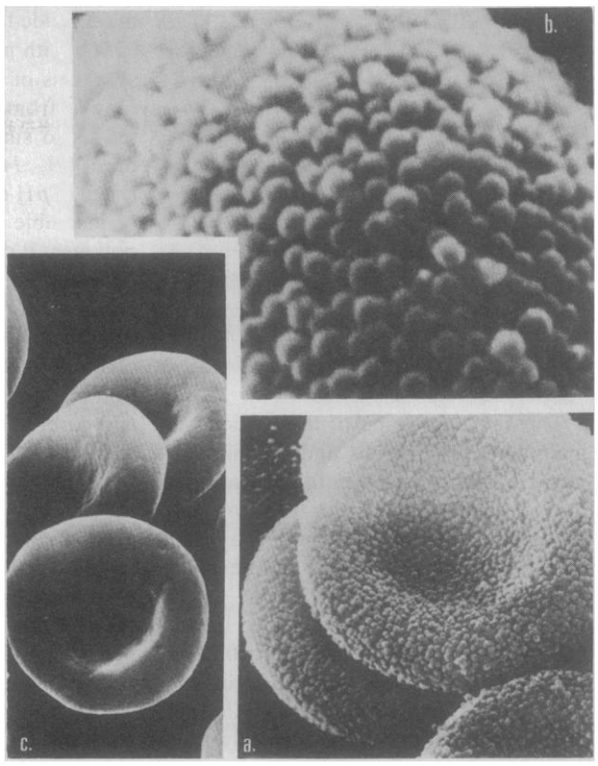


Fig. 1. An SEM photomicrograph of (a) a human red blood cell labeled with fluorescent microspheres 60 nm in diameter (× 10,000), prepared by the emulsion polymerization technique. The indirect labeling method was used; red blood cells were sensitized with rabbit antiserum to red blood cells. The red blood cells were then reacted with microspheres coated with goat antibodies to rabbit serum. A detailed procedure for cell labeling has been published (*9*). (b) Higher magnification than that of (a); (c) control, that is, nonspecific antiserum bound to microspheres was used (×7000); PGL microspheres gave similar results.

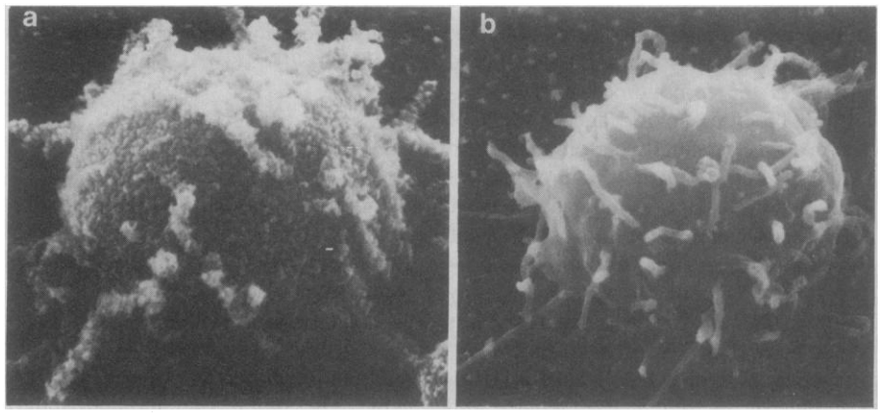


Fig. 2. An SEM photomicrograph of (a) labeled and (b) unlabeled mouse spleen lymphocytes (*9*). (a) Mouse spleen lymphocyte treated first with rabbit antiserum to mouse lymphocytes and then with fluorescent microspheres which had been conjugated to goat antibodies against rabbit immunoglobulin. The microsphere diameter was about 75 nm. In the control (b), a nonspecific rabbit serum was substituted for rabbit antiserum to mouse lymphocytes (×10,000).

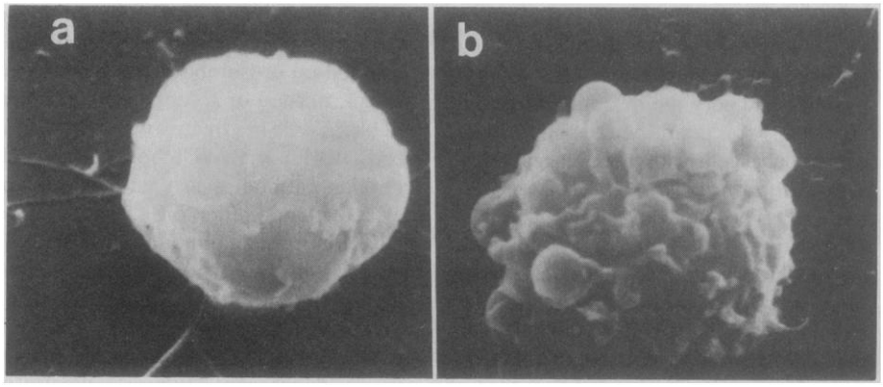


Fig. 3. An SEM photomicrograph of a human lymphocyte labeled with PVP microspheres 800 nm in diameter that had been conjugated with human immunoglobulin G. This procedure detects Fc receptors (*13*). (a) Control, that is, the microspheres were not treated with immunoglobulin G. (b) Labeled cell.

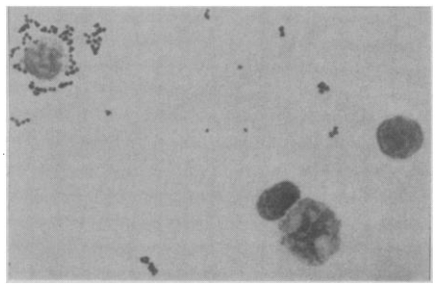

Fig. 4. Light microscope photomicrograph of murine spleen cells labeled with 800-nm microsphere conjugates (*15a*).

The size and shape of the particles is maintained in aqueous and organic solvents commonly used in the preparation of biological samples for electron microscopy. This stability is largely a result of the cross-linked nature of the polymeric matrix.

*Ionizing radiation*. The design of microspheres of various sizes and containing functional groups was based on the observation that $^{60}Co$ irradiation of HEMA dissolved in water yielded water-insoluble spherical particles. Subsequent studies (*11*) showed that the size and properties of these particles vary considerably as a function of, for example, monomer concentration, the functional groups of the comonomer, and concentration of cross-linking agent. Most of the described results were obtained with copolymer systems in the presence of polyethylene oxide which acts as a steric stabilizer and permits the use of relatively high concentrations of monomers (up to about 20 percent). Highly fluorescent and stable microspheres could be obtained by an addition of a fluorescent monomer, such as dansyl allylamine or an adduct of allylamine and fluorescein isothiocyanate, to the comonomer mixture. Parameters affecting size, aggregational properties, and concentration of functional groups have been described (*11*, *12*).

The application of ionizing radiation to isomeric vinylpyridines yielded microspheres varying in size between 30 and 10,000 nm. Copolymers of 4-vinylpyridine and acrylamide could be used to chemically bind antibodies and subsequently label cells with these reagents (*13*).

*Polyglutaraldehyde (PGL) microspheres*. The derivatization of microspheres prepared either by the emulsion or $^{60}Co$ irradiation technique generally requires several intermediate steps. For example, in one procedure the cyanogen bromide method was used to react hydroxyl groups, present on the surface of microspheres, with diaminohexane to extend an "arm" carrying an amino group. Then the reaction with glutaraldehyde yielded an aldehyde function for reaction with proteins and antibodies. Our synthesis of PGL microspheres in sizes ranging from about 50 to 1500 nm permitted us to simplify the derivation procedure (*13*, *14*). When prepared in the range of *p*H of 7 to 13.5, whether soluble or insoluble in water, PGL is a reactive polymer. It contains variable numbers of hydrated and nonhydrated aldehyde groups that may or may not be adjacent to an ethylenic linkage, and it is also characterized by a variable concentration of primary and secondary hydroxyl and carboxyl functions. The presence of secondary hydroxyl groups is explained by an incomplete aldol condensation and that of primary hydroxyl and carboxyl group by the occurrence of a Cannizzaro reaction for which ample evidence was obtained. Air oxidation of aldehyde during and after the polymerization at high *p*H may yield additional carboxyl functions (*15*).

The high reactivity of PGL, whether in solution, in solid state, or in the form of water-insoluble fluorescent or magnetic microspheres, offers possibilities for important applications of PGL as a new immunoreagent in biology and biochemistry.

## Cell Labeling

Antibodies bound to highly fluorescent microspheres can serve as fluorescent markers to detect specific cells when bound to antigens on the cell surfaces and examined with a fluorescent microscope. Cells coated with such immunomicrospheres are intensely fluorescent. The same immunomicrospheres can also be used as scanning electron microscope (SEM) markers to detect and map the distribution of a variety of different cell surface antigens at a much higher magnification than is possible in the fluorescent microscope.

The activity and specificity of these microsphere reagents can be readily determined on red blood cells. In this test system, unfixed or aldehyde-fixed human red blood cells are treated with rabbit antiserum to human red blood cells which contains antibodies against a variety of surface components on the red blood cells. Fluorescent immunomicrospheres coated with goat antibodies directed against the rabbit antibodies are then used to visualize the red blood cell antigens (Fig. 1). These labeled cells are intensely fluorescent. Figure 2 illustrates murine lymphocytes labeled in a similar manner. Figure 3 shows the labeling of Fc receptors on lymphocytes by means of 800-nm PVP microspheres. Lymphocytes may also be labeled with $^{60}Co$ radiation-synthesized microspheres that are large enough to be seen in the light microscope (*15a*) without fluorescence (Fig. 4). Immunomicrospheres can also be used to identify subpopulations of cells within tissues. An SEM photomicrograph of the surface of a mouse spleen slice is shown in Fig. 5. The labeled cells are assumed to be B cells.

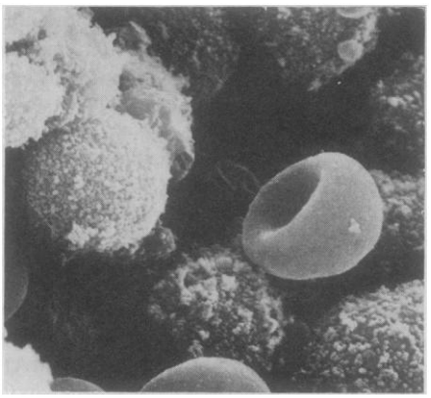

Fig. 5. An SEM photomicrograph of a mouse spleen tissue slice lightly fixed with glutaraldehyde, sensitized with rabbit antiserum to mouse immunoglobulin G and labeled with 100 nm microspheres conjugated with goat antibodies to rabbit immunoglobulin. Labeled cells are presumed to be B cells. See (*9*) for details of similar procedures.

## Fluorescent Immunomicrospheres in the Analysis and Separation of Cells

One of the interesting and useful properties of many different types of cells is the display of different molecules on their surfaces. For example, some cells such as the B cells and T cells (lymphocytes) look very much the same when examined in the standard way used for blood cell counting. Nevertheless, there is an important need to analyze and study different types of lymphocytes. In the past, both basic research in immunology and important clinical studies have been restricted because of the lack of a suitable means for analysis and separation of these various types of cells.

Recently several powerful instruments that are able to analyze cells as they flow past a microscopic beam of light have become commercially available (*16*). Low-angle light scattering provides information on the cell size and, at the same time, fluorescent emission can be monitored at two different wavelengths. These properties are then used both in the analysis of the sample of cells and also as the basis for the separation of specific cell types. Antibodies, each labeled with one or two molecules of fluorescent

dye, have been used for labeling specific cell types. However, at least 300 dye molecules must be bound to each cell to permit detection. This limitation has generated interest in the use of fluorescent immunomicrospheres in these applications. Since hundreds or thousands of dye molecules are incorporated into each fluorescent microsphere, the instruments can easily detect a single sphere. Accordingly, cells that display a relatively small number of the molecules of interest can be tagged with fluorescent immunomicrospheres and analyzed in the fluorescence-activated cell-sorting instruments (*16*).

The greatly increased intensity of fluorescence emitted by cells labeled in this manner should also allow the design of instruments with simplified optics that are, nevertheless, able to detect the fluorescent light emitted by the new reagents. Simpler low-cost instruments would be of interest in several clinical cell-typing applications. It also seems likely that similar routine instrumental analyses will permit rapid detection and identification of various microorganisms of clinical importance. Current methods such as cell culture often require several days and are relatively costly.

### Electrophoretic Cell Separation

Another method of cell separation that is of interest is based on the mobilities of cells in an electric field. Resolution in electrophoretic separation of cell subpopulations, currently limited by finite and often overlapping mobility distributions, may be significantly enhanced by immunospecific microsphere labeling of target populations. The electrophoretic mobility of fixed human red blood cells immunologically labeled with polymeric 4-vinylpyridine (PVP) or PGL microspheres was altered to a considerable extent, that is, from $-1.07$ $\mu$m sec$^{-1}$V$^{-1}$ cm for unlabeled cells to $-0.65$ $\mu$m sec$^{-1}$ V$^{-1}$ cm for human red cells labeled with PVP microspheres and $-0.62$ $\mu$m sec$^{-1}$ V$^{-1}$ cm for human red cells labeled with PGL microspheres. This characteristic was used in a preparative-scale electrophoretic separation of cells by continuous flow electrophoresis.

Two model systems were used: (i) a mixture of labeled and unlabeled human red blood cells and (ii) a mixture of labeled human red blood cells and unlabeled turkey red blood cells. The labeled cells of both systems (ratio 1:1) were efficiently separated (*17*) by means of a continuous flow electrophoresis instrument (Beckman Instruments, Inc.) (*18*).

### Magnetic Cell Separation

A new development of importance for cell sorting is the synthesis of functional microspheres containing $Fe_3O_4$. Initial investigations (*19*) show that both T and B cells labeled with magnetic immunomicrospheres can be efficiently separated from mixed cell populations by means of a magnet.

The separation of human red blood cells labeled with magnetic PGL microspheres was achieved as follows. Mixtures of human red blood cells with the following ratios of unlabeled to labeled cells were prepared—1:1, 7:1, and 9:1. The mixtures (10 milliliters) were gently stirred in a glass vial fitted with a simple horseshoe magnet (300 gauss). At the end of 2 hours, cells that were not attracted to the vessel walls were isolated. Cells attracted by the magnet were diluted with 10 ml of phosphate-buffered saline, and the magnetic separation was repeated. Scanning electron microscope examination showed that more than 95 percent of unlabeled cells could thus be separated from all three synthetic mixtures (*14*).

The evidence obtained so far with model cell systems indicates that magnetic PGL immunomicrospheres of desirable sizes can be conjugated with antibodies in a simple and convenient manner, therefore offering a potential for large-scale immunological cell sorting.

### Other Present and Potential Applications

*Electron-opaque microspheres.* Other types of reagents may be devised to facilitate the examination of cell surfaces. For example, sufficiently small electron-opaque metal-containing microspheres should provide higher spatial resolution of cell surface features than is now possible in the electron microscope. It is anticipated that gold or platinum particles incorporated in a polymeric matrix with functional groups on the surface capable of forming a covalent bond with proteins would offer more stable labels than gold particles with physically adsorbed antibodies, which are now used for cell labeling. Gold and platinum microspheres can be very simply obtained by reaction of chlorauric or chloroplatinic acid, respectively, with PVP microspheres (*12*) or by polymerization of suitable monomers in the presence of freshly formed small colloidal gold particles.

The PVP microspheres that had been reacted with chloroplatinic acid were injected intravenously into mice. They were then easily detected in electron microscope photomicrographs of spleen, liver, and kidney tissue sections (Fig. 7). Hence these reagents offer the possibility of analysis, by ultrastructural techniques, of localized pathologic and pharmacologic processes in experimental systems. They may also yield information in studies of the pathology of in-

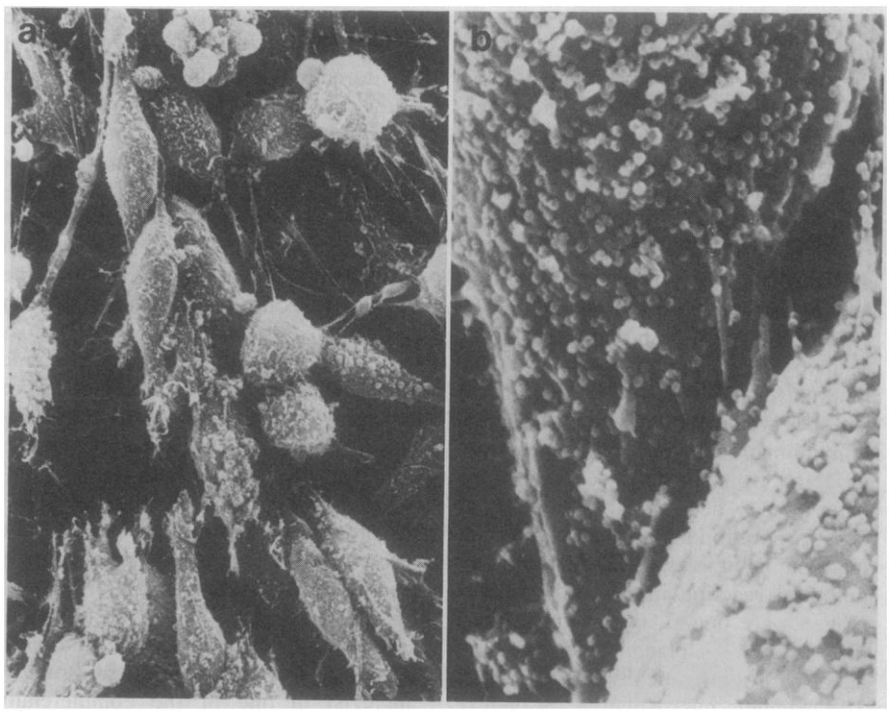


Fig. 6. Tumor cells tagged with fluorescent microspheres 60 nm in diameter. Antibodies reactive against these cultured fibrosarcoma cells (*20, 21*) were prepared by immunizing normal syngenic C3H mice with live tumors. The labeling procedure was essentially the same as in (*9*). (a) × 1,000. (b) ×11,000.

jurious agents that may be introduced in vivo, such as particular lung irritants (*15a*).

*Immunofluorescent assays*. Radioisotopes such as $^{125}I$ are often coupled to antibodies or antigens so that an immunological reaction can be monitored. For example, after $^{125}I$-labeled antibodies react with specific antigens on cell surfaces, the cells may be freed of excess antibody; and the extent of the reaction can be monitored in a gamma counter. The intensely fluorescent immunomicrosphere reagents can be used in much the same way, but a fluorimeter instead of a gamma counter is used to monitor the reaction. In many applications, the speed and sensitivity of the assays with fluorescent microspheres are greater than in conventional radioimmune assays. The reason for the increase in sensitivity of fluorescent beads over radioactive labels is easily understood when we consider that, upon illumination, a single fluorescent immunomicrosphere emits a large number of photons in a fraction of a second and that these are easily detected with appropriate instruments. In contrast, an antibody labeled with an $^{125}I$ atom has a theoretical maximum chance of 50 percent of being detected in 63 days (the half-life of $^{125}I$).

Many millions of radioimmunoassays are performed each year in order to ensure that blood used for transfusion does not carry the hepatitis antigen. A common form of this assay involves an immunological reaction with molecules bound to a bead several millimeters in diameter, and is monitored by measuring the $^{125}I$ radioactivity remaining attached to the bead after the immunological reaction. It seems likely that small magnetic beads might replace the large bead used in this assay and, further, that fluorescent or luminescent immunomicrospheres might be bound to beads or other surfaces in much the same way as $^{125}I$-labeled antibodies are now used. This type of assay could be automated with relative ease, and many of the problems inherent in the large-scale use of radioisotopes would be eliminated. Magnetic immunomicrospheres of a composition different from those discussed above have already been used by the Technicon Corporation as a means of automating radioimmune assays. After an immunological reaction in which $^{125}I$-labeled reagents are bound, the magnetic particles are held in place while excess reagents are automatically washed away. The magnetic microspheres are then released to flow through a gamma counter.

Many other variations of radioimmune assays have been devised, and appropriate immunomicrospheres might replace isotopes in a number of cases. For example, many radioimmune assays are performed with test tubes or wells coated with antigen. Radioactively labeled reagents then react with the walls of these containers. After completion of the reaction and appropriate washing, the radioactivity remaining on the container is counted. In a test of this approach, we found that it was possible to coat a small capillary tube with antigen and bind antibody-coated fluorescent microspheres to the walls. The walls of the tube became intensely fluorescent. The bound microspheres could be eluted and monitored in a fluorimeter or detected in situ.

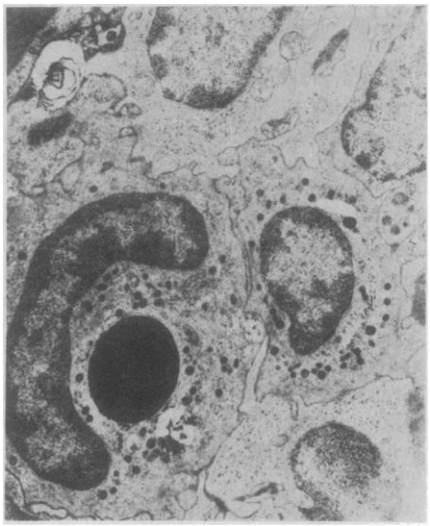

Fig. 7. Electron micrograph showing phagocytosis of PVP platinum beads, 3500 nm in diameter, by a mouse spleen macrophage (*15a*).

*Tumor biology*. Most, if not all, tumors can be detected by the host immune system. In the case of some murine tumors now under study, it has been shown that a healthy immune system can destroy tumors, and suppression of the immune response leads to tumor growth and death of the animal (*20, 21*). Tumors were used to immunize healthy syngenic mice. The antibodies produced were then reacted with tumor cells grown either in vivo or in tissue culture. Appropriately coated, highly fluorescent immunomicrospheres were then used to detect the presence of mouse antibodies on the tumor cells. These assays are routinely performed by monitoring fluorescence, but the labeled cells may also be observed by SEM (see Fig. 6). Tumor cells labeled in this way may also be rapidly analyzed in one of the commercially available flow-through instruments described above which quantitate both light scattering of all cells and the fluorescence of labeled cells. The analysis of specific populations of tumor cells and the study of molecular markers on cell surfaces is now undergoing a revolutionary advance due to the new techniques for production of high titer monoclonal antibodies. The availability of both monoclonal antibodies and the immunomicrophore reagents is facilitating the isolation and study of the antigens found on tumor cells.

We can anticipate ways in which various types of immunomicrospheres might be used in early cancer detection, diagnosis, and perhaps, in new types of therapies. For example, drug-containing microspheres have already been synthesized which may be useful for destroying specific target cells. The surprising breadth and diversity of uses which numerous scientists have already found for these new reagents are indications that future applications could be even more interesting.

### References and Notes


1. W. A. Wagner, *Res. Immunochem. Immunobiol.* **3**, 185 (1933).
2. B. Pernis, L. Forni, L. Amante, *J. Exp. Med.* **132**, 1001 (1970); E. Rabellino, S. Colon, H. Grey, E. Unanue, *ibid.* **133**, 156 (1971); R. S. Molday, Illinois Institute of Technology Research Int./SEM (1977), vol. 2, pp. 59–74; M. Karnovsky, E. Unanue, J. Leventhal, *J. Exp. Med.* **136**, 907 (1972); T. Aoki, E. Boyse, L. Old, E. de Harven, U. Hammerling, H. Wood, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 569 (1970).
3. N. L. Wagner, *Adv. Immunol.* **191**, 67 (1974).
4. S. S. Froland and J. B. Natvig, *Transplant. Rev.* **16**, 114 (1973); M. Jondal, H. Wigzell, F. Aiuti, *ibid.*, p. 163.
5. J. P. Brockes, K. L. Fields, M. C. Ratt, *Brain Res.* **165**, 105 (1979).
6. G. Roth, *J. Immunol. Methods* **18**, 1 (1977).
7. P. Cuatrecasas, *J. Biol. Chem.* **245**, 3059 (1970).
8. T. L. Goodfriend, L. Levine, G. D. Fasman, *Science* **144**, 1344 (1964).
9. R. S. Molday, W. J. Dreyer, A. Rembaum, S. P. S. Yen, *J. Cell Biol.* **64**, 75 (1976).
10. S. P. S. Yen, A. Rembaum, R. S. Molday, W. J. Dreyer, *Emulsion Polymerization* (ACS Symposium Series, Washington, D.C., 1979), No. 24, p. 236.
11. A. Rembaum *et al.*, *Macromolecules* **9**, 328 (1976).
12. A. Rembaum, S. P. S. Yen, R. S. Molday, *J. Macromol. Sci. Chem.* **13** (No. 5), 603 (1979).
13. A. Rembaum, S. Margel, J. Levy, *J. Immunol. Methods* **24**, 239 (1978).
14. S. Margel, S. Zisblatt, A. Rembaum, *ibid.* **28**, 341 (1979).
15. S. Margel and A. Rembaum, *Macromolecules*, **13**, 19 (1980).

15a. I. L. Gordon, N. J. Dreyer, S. P. S. Yen, A. Rembaum, *Cell. Immunol.* **28**, 307 (1977).

16. H. R. Hulett, W. A. Bonner, R. G. Sweet, L. A. Herzenberg, *Clin. Chem.* **19**, 813 (1973).
17. A. J. K. Smolka, S. Margel, B. H. Nerren, A. Rembaum, *Biochim. Biophys. Acta.* **588**, 246 (1979).
18. A. Strickler and T. Sachs, *Ann. N.Y. Acad. Sci.* **209**, 497 (1973).
19. R. S. Molday, S. P. S. Yen, A. Rembaum, *Nature (London)* **268**, 437 (1977).
20. M. L. Kripke, *J. Natl. Cancer Inst.* **53**, 133 (1974).
21. C. W. Spellman and R. A. Daynes, *Cell. Immunol.* **38**, 25 (1978).


22. This article represents one phase of research carried out by the Jet Propulsion Laboratory, California Institute of Technology, under NASA contract NAS7-100 sponsored by the National Aeronautics and Space Administration and by NIH grants 1R01CA20668, GM06965, and AI 14059.