Saccharides Mediate the Attachment of Rat Macrophages to Bone In Vitro

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ABSTRACT Macrophages (MØ) are multipotential cells capable of giving rise to osteoclasts and of resorbing bone. Since both of these processes are ultimately dependent upon the attachment of cells to a mineralized bone surface, we have examined in this study the mechanism by which such attachment is achieved. The data show that elicited rat peritoneal MØ bind to bone in a temperature-dependent and -saturable manner with half-maximal attachment occurring within 10 min at 37°C and reaching a plateau by ~60 min. The kinetics of binding are essentially the same whether devitalized bone particles or viable calvaria are used as a substrate. The attachment of MØ to bone is inhibited by some sugars (e.g., *N*-acetylgalactosamine, thiogalactoside, β-lactose), fetuin and asialofetuin, and by pretreating the bone with periodate. Binding is also significantly reduced when MØ are preincubated with tunicamycin and swainsonine at nontoxic concentrations sufficient to inhibit or alter glycosylation. On the other hand, exposing the cells to neuraminidase increases the capacity of MØ to bind to bone. Collectively, our observations indicate that the attachment of MØ to bone is a highly regulated process and is mediated, at least in part, by saccharides located on both the cell and the bone surface.

INTRODUCTION

Despite its importance as a major functional component in mineral homeostasis and skeletal modeling and remodeling, osteoclastic bone resorption remains poorly understood. This lack of understanding no doubt reflects, at least in part, the complexity of the experimental systems that have been used to study the phenomenon. Traditionally, these models have used either intact animals (1) or organ cultures of bone rudiments (2) which, by virtue of their heterogeneous, multicellular composition make identification of the specific cell type responsible for a particular biological event difficult, if not impossible. Moreover, the physical intimacy of the cells and bone matrix in animals or fetal rudiments generally precludes discrete analysis of the roles these two major constituents play in regulating osteoclast formation and resorptive activity.

Recently, we developed a model for the study of bone resorption based upon the use of mononuclear phagocytes (monocytes and macrophages) as a paradigm of the resorbing cell (3). This paradigm follows from the established familial relationship of the mononuclear phagocyte (MØ)[1] to the osteoclast (4) and the fact that the MØ, in vitro, resorbs bone in a manner morphologically and histochemically reminiscent of the osteoclast in vivo (8).

Our previous work on MØ-mediated bone resorption has repeatedly underscored the fact that bone resorption is initiated by, and dependent upon, intimate contact between the cell and the bone surface (3, 9). Similarly, observations by us (10, 11) and other investigators on osteoclast development in vivo (12, 13) indicate that contact between the osteoclast precursor (a cell belonging to the MØ family) and mineralized bone is also essential for the initiation and completion of osteoclast differentiation. This realization of the pivotal role played by cell-matrix interaction in osteoclast formation and resorptive activity raises the question of how MØ recognize and attach to bone surfaces.

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Received for publication 30 September 1982 and in revised form 20 April 1983.

[1] *Abbreviations used in this paper:* α-10, α-MEM supplemented with 10% fetal calf serum; α-MEM, Eagle's minimal essential medium, α-modification; MØ, macrophage(s); α-MOPS, MEM buffered with 3(*N*-morpholino) propane sulfonic acid; SW, swainsonine; TM, tunicamycin.

In this communication, the basic, quantitative characteristics of MØ-bone attachment are described, and evidence is presented that recognition and binding are mediated, at least in part, by saccharides located on both the cell membrane and the bone surface.

MATERIALS AND METHODS

Cells

100–150-g male rats were injected, i.p. with sterile 10% thioglycollate and, 3 d later, subjected to peritoneal lavage. The cells in the lavage fluid were washed by centrifugation and suspended in serum-free Eagle's minimal essential medium (α-modification; α-MEM) buffered to pH 7.4 with 3 (*N*-morpholino) propanesulfonic acid (α-MOPS). The cell suspension, containing ~70% elicited MØ, was stored briefly on ice, until plated into multiwell dishes (KC Biological, Kansas City, MO).

Binding assays

Particle binding. The methods used to prepare bone particles and obtain cells are described in detail in reference 3. Briefly, long bones were excised from $^{45}CaCl_2$-injected rats (700 μCi total/animal), stripped free of soft tissue and mechanically fractured to expedite removal of marrow. The bone fragments were rinsed thoroughly in sterile saline, dehydrated at 40°C, and ground into particles using a Spex mill (Spex Industry, Inc., Metuchin, NJ). The particles were then passed successively through nylon mesh screens (Cistron Corp., Lebanon, PA) of 45- and 23-μm pore size, and the retentate of the second sieving collected and UV-sterilized for use in binding assays. The relatively large size of particle precludes their internalization by MØ.

Elicited peritoneal cells were suspended in α-MOPS to a concentration of 5×10^5/ml and pipetted into multiwell dishes at 0.2 ml/6-mm diam well. After 1 h of incubation at 37°C, the cultures were rinsed and the medium replaced with bicarbonate-buffered α-MEM supplemented with 10% fetal calf serum (α-10). The dishes were incubated for an additional 20–23 h and rinsed again to remove the nonadherent cell fraction. 60–70% of the exudate cells remain attached and, of these, ~98% were MØ as assessed by esterase staining and phagocytosis.

Bone particles were suspended in α-MOPS at concentrations ranging from 0.5 to 10 mg/ml (1 mg/ml in the typical experiment) and were introduced into the cultures in 0.2-ml aliquots. The dishes were incubated at the specified temperature in air for periods up to 2 h, and the nonadherent particles removed by immersing and agitating the culture dishes in three successive changes of phosphate-buffered saline (PBS). Concentrated trichloroacetic acid (TCA) was then added to each well to solubilize the residual, attached particles and the resultant, radioactive slurry dissolved in scintillation fluid (Scintiverse, Fisher Scientific Co., Pittsburgh, PA) for counting. The net, cell-associated binding of particles was determined by subtracting the disintegrations per minute obtained from cell-free wells from counts derived from cultures containing MØ monolayers.

Calvarial binding. Elicited peritoneal exudate cells in α-MOPS were plated into 60-mm tissue culture dishes and rinsed after 1-h incubation to remove the nonadherent fraction. α-10 was then added to the dishes and, after an additional 20–23 h of culture, the plates were rinsed again to remove the remaining nonadherent cells and the medium replaced with Ca^{++}/Mg^{++}-free PBS at 4°C. 20 min later, the attached MØ were brought into suspension by gentle scraping with a rubber policeman. The MØ were then labeled for 1 h at 37°C with $Na^{51}CrO_4$ (sp act 250–500 μCi/mg Cr) (Amersham Corp., Arlington Heights, IL) (1 μCi/10^6 cells in α-MOPS), washed three times with cold α-MOPS and suspended in the same medium at 2×10^6 cells/ml. 0.1-ml aliquots of the cell suspension were pipetted onto the endocranial surface of freshly dissected rat calvaria. The latter are obtained from 7-d rat pups and were freed of their inner periosteum by mechanical stripping. The calvaria were then placed, concave-side up, in multiwell culture dishes and the cells added.

Cell number

The number of adherent cells was determined by the methylene blue-binding technique described by Goldman and Bar-Shavit (14). In brief, cells were fixed overnight in 2.5% formaldehyde, washed in 0.1 M borate buffer (pH 8.5), and incubated for 10 min in methylene blue (1 mg/ml in borate buffer). The cells were then washed extensively in buffer to remove excess dye and extracted with 0.1 N HCl at 37°C for 40 min to solubilize the cell-associated methylene blue. The eluate was diluted 1:6 with water and measured spectrophotometrically at 660 nm. Dye binding was proportional to cell number.

Protein synthesis and glycosylation

Protein synthesis and glycosylation were assessed from the incorporation of [^{3}H]leucine (sp act 58.5 Ci/mM, New England Nuclear, Boston, MA) and [^{3}H]mannose (sp act 47 Ci/mM, New England Nuclear), respectively. Cells were incubated for 3 h in isotope, rinsed three times in 10% TCA and two times in a mixture of ethanol and ether (3:1 vol/vol). The insoluble fraction was digested in NaOH, dissolved in Scintiverse, and counted.

RESULTS

Characteristics of MØ-bone attachment. The binding of bone by monolayers of elicited MØ is a saturable process with half-maximal attachment occurring at particle concentrations of ~0.35 mg/6-mm diam well and saturation at ~1.25 mg/well (Fig. 1). By contrast, nonspecific binding of particles to cell-free wells is not saturable at concentrations up to 2 mg/well, increases linearly with particle concentration, and does not exceed 3–4% of the total binding observed in the presence of MØ monolayers (Fig. 1). The attachment of isotopically labeled particles to MØ is diminished in a dose-dependent fashion by the concurrent addition of excess nonradioactive bone (Fig. 2).

Bone particle binding by MØ is temperature dependent, with both the rate and magnitude of binding varying markedly over the range of 4° to 37°C. At 37°C, half-maximal binding occurs within 10 min and

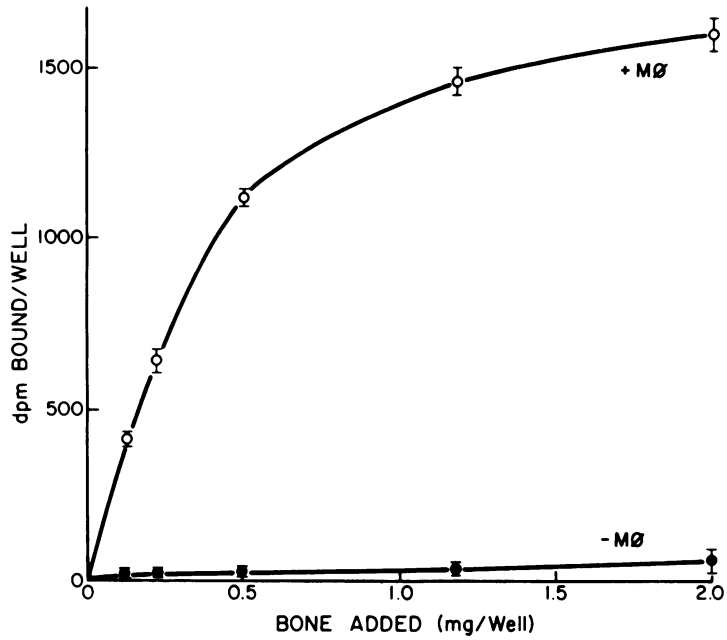

FIGURE 1 Dose-dependent binding of radiolabeled bone particles to MØ monolayers. Gradual addition of ^{45}Ca-labeled particles shows half-maximal attachment at ~0.3 mg/6-mm diam well and saturation at ~1.25 mg/well. Assay run for 2 h at 37°C with 1 × 10^5 cells/culture. Each point represents the mean±SE of six replicate cultures.

maximal attachment by 60 min (Fig. 3). At the latter time and temperature, ~50% of the bone particles added to the wells are bound to the MØ monolayers. In contrast, at 4°C, half-maximal binding is not observed until 30 min of incubation and by 60–120 min, only 10–12% of the particles are attached (Fig. 3).

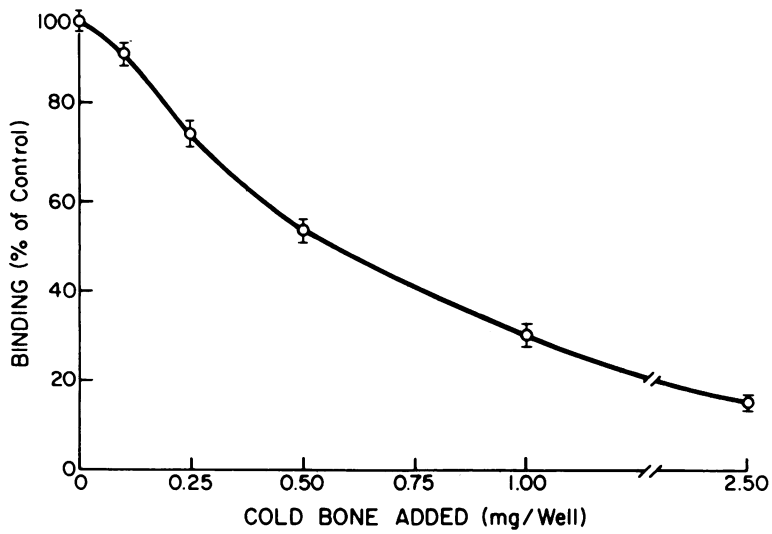

FIGURE 2 Competition between radiolabeled bone particles and unlabeled bone. ^{45}Ca-labeled particles (0.5 mg/well) were premixed with the indicated amount of unlabeled bone and added to cultures containing 1 × 10^5 MØ. Note the 1:1 correlation between the decline in radioactivity (percentage of control level) and the incremental addition of cold particles. Incubation period, 1 h at 37°C. Each point represents the mean±SE of six replicate determinations.

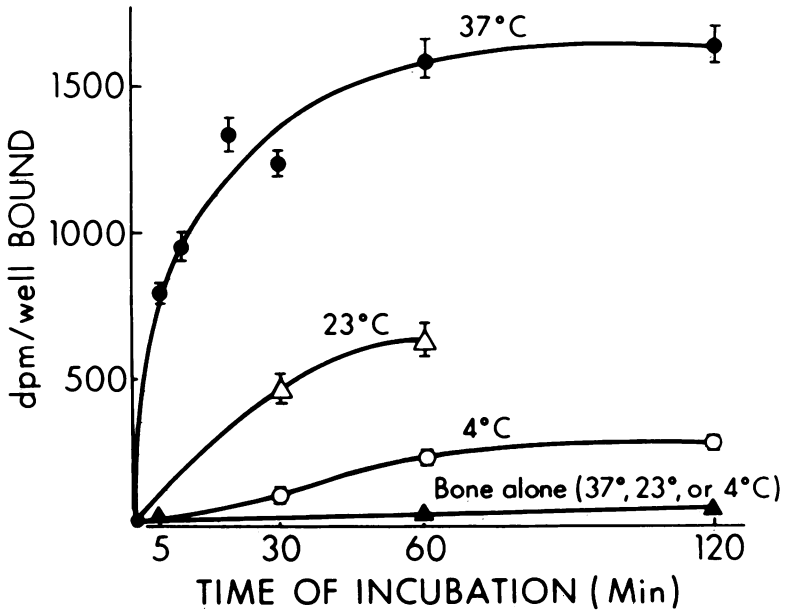

FIGURE 3 Time course of bone particle binding to MØ as a function of temperature. Note that the rate and extent of attachment is significantly greater at higher than at lower temperatures and that at 37°C, half-maximal binding is achieved within 10 min and is completed within 60 min. 0.2 mg ^{45}Ca-labeled bone was added to each well; sp act 6,150 dpm/0.2 mg. Each value represents the mean±SE from six replicate cultures.

Similarly, preincubation of the MØ monolayers with the metabolic inhibitor NaN_3 (10^{-4} M for 4 h at 37°C) reduces the fraction of bone particles bound to ~20% (data not shown). The difference in the percentage of particles attached at low temperature (~10%) vs. that observed with azide treatment (~20%) suggests that the effect of cold is not only a matter of reduced metabolic activity but also the result of reduced fluidity of the plasma membrane.

The production of bone particles not only contributes to their devitalization but also results in free surfaces that are likely to be heterogeneous with regard to the degree of matrix maturation, i.e., some particle surfaces are likely to be covered with osteoid (unmineralized bone) while others will be coated with fully mineralized matrix. To circumvent this difficulty, and to independently confirm the binding kinetics with bone particles, a second protocol was developed in which ^{51}Cr-labeled MØ were allowed to bind to the stripped endocranial surface of freshly dissected rodent calvaria. As can be seen in Fig. 4, the kinetics of attachment to this more uniform, still viable bone matrix are strikingly similar to those observed in the bone particle assay.

Role of carbohydrates (oligosaccharides) in MØ-bone attachment. The antibiotic tunicamycin (TM) has been shown to inhibit the glycosylation of proteins in a number of different biological systems (15, 16). Exposure of MØ to TM (Sigma Chemical, St. Louis, MO) at a concentration (0.4 μg/ml) sufficient to inhibit glycosylation (i.e., [^{3}H]mannose incorporation) but not

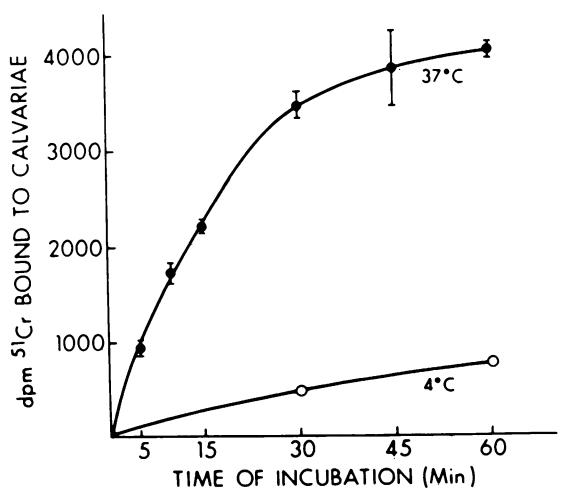

FIGURE 4 Kinetics of attachment of radiolabeled MØ to rat calvaria. ^{51}Cr-labeled cells at 1 × 10^6 cells/ml were added in 100-μl aliquots to the stripped, endocranial surface of freshly dissected calvaria and incubated at 37° and 4°C. At 37°C, adherence is clearly evident by 5 min and is nearly completed by 60 min. Sp act, 9,600 dpm/10^5 cells. Each point represents the mean±SE of six replicate cultures.

protein synthesis (i.e., [^{3}H]leucine incorporation) or cell survival (Fig. 5), significantly reduces the ability of MØ to bind bone particles (Fig. 6 *A*). These inhibitory effects of TM on mannose incorporation and binding are reversible (Fig. 6 *A*) and demonstrable in the presence of the protease inhibitor leupeptin (Sigma Chemical Co.) (Fig. 6 *B*).

Swainsonine (SW) is a compound that inhibits α-mannosidase activity and the hydrolysis of terminal mannose residues from initially high mannose-containing glycoproteins (17). It would be expected, therefore, that if membrane glycoproteins are important in cell-bone attachment, treating MØ with SW should alter the binding process. In fact, SW-treated MØ (0.2 μg/ml for 16 h at 37°C) are suppressed in their ability to bind bone to the same extent as TM-treated cells (data not shown). This observation further confirms a role for oligosaccharides in cell-bone attachment and suggests that sugar residues other than mannose are most essential in the attachment process.

A more direct approach to the possible role of saccharides in the attachment of MØ to bone involves the use of simple sugars and aminosugars (Sigma Chemical Co.) as competitive blocking agents in the binding assay. As can be seen in Fig. 7, sugars fall into three main categories; those with no effect on MØ-bone attachment (xylose, and not illustrated, galactose and sucrose), those moderately inhibitory (fucose, mannose), and those that strongly curtail MØ-bone binding (*N*-acetylglucosamine, *N*-acetylgalactosamine, and β-lactose reduce attachment by ~60%). Similar patterns of inhibition are obtained using both the particle binding and calvarial assays, but are not observed in the attachment of MØ to plastic. In fact, in the latter case, sugars do not significantly suppress binding (data not shown). Finally, no inhibition of cell-bone binding is observed if either cells or bone particles are preincubated with any sugar and then washed before the binding assay (data not shown). Thus, it appears that (*a*) saccharides are involved in the attachment of cells to bone; (*b*) that some sugars are more likely to be involved than others; and (*c*) MØ attachment to bone differs fundamentally from binding to a nonbiological surface.

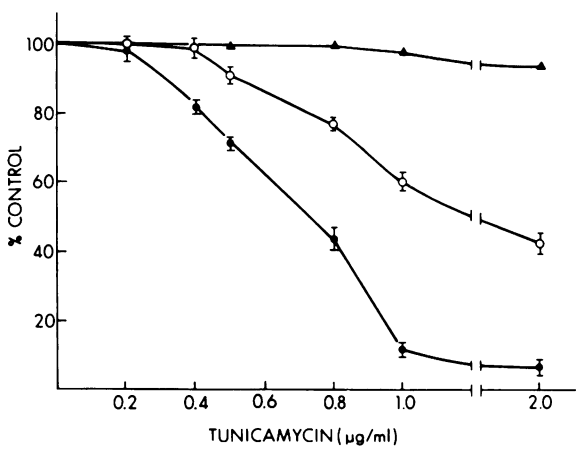

FIGURE 5 Effect of TM on MØ cell viability, protein synthesis, and glycosylation. Cells were incubated for 18 h in the specified concentrations of TM with [^{3}H]mannose or [^{3}H]leucine present for the last 3 h. The incorporation of the two labeled compounds was determined from TCA-insoluble fractions and taken to indicate glycosylation and protein synthesis, respectively. Cell number (▲) was determined from parallel unlabeled cultures using the methylene blue-binding technique. At 0.4 μg/ml, TM significantly curtails [^{3}H]mannose (●) ($P < 0.001$) but not [^{3}H]leucine (○) incorporation. TM does not affect cell viability at any concentration up to 2.0 μg/ml. Each point represents the mean±SE of three replicate cultures.

It is of interest that β-lactose and thiogalactoside, which are among the most potent competitors of MØ-bone attachment, also inhibit the binding of the circulating fetal glycoprotein, fetuin, and particularly its asialo-derivative to cells (18). Because such inhibition by the glycoproteins probably represents recognition by a commonly occurring membrane lectin, one might expect fetuin and asialofetuin to be competitive inhibitors of MØ-bone binding. In fact, MØ-bone binding is inhibited by fetuin and especially asialofetuin (Sigma Chemical Co.) in a dose-dependent manner (Fig. 8). A similar inhibition of binding is also observed when the cells, but not the bone, are incubated with asialofetuin before the assay (data not shown). On the other hand, bovine serum albumin, which is a nonglycosylated protein, does not affect MØ-bone binding even when present throughout incubation (data not shown).

The terminal carbohydrate residue of most glycoproteins is sialic acid, which contributes to the net charge on the cell-surface (19) and often serves to block optimal oligosaccharide-lectin interaction (20). The involvement of this carbohydrate residue in MØ-bone binding was determined by preincubating cells with neuraminidase (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) to remove terminal sialic acid residues from membrane glycoproteins. Fig. 9 shows that such pretreatment results in a subsequent increase in the binding of bone particles by MØ. On the other hand, similar exposure of bone particles and calvaria to the enzyme fails to alter binding activity (Fig. 10).

Periodate (Sigma Chemical Co.) is a potent chemical oxidant of carbohydrates (21) and, as such, might be expected to alter cell-bone attachment if matrix-associated carbohydrates are involved in the binding process. That this is indeed the case is shown in Fig. 10, where the data demonstrate that pretreatment of bone particles and calvaria with periodate results in

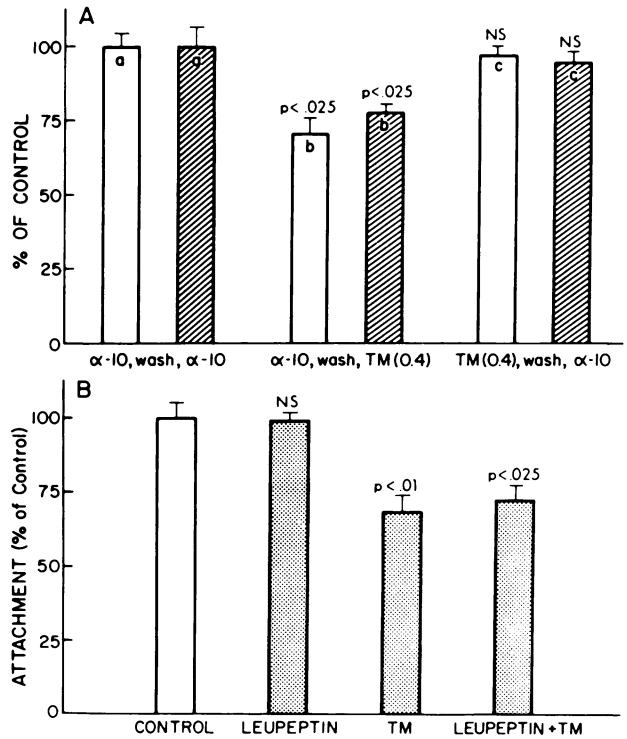

FIGURE 6 Leupeptin resistance and reversibility of the TM effect on MØ. (*A*) Bone binding levels and [^{3}H]mannose incorporation (■) were assessed in control cultures (α-10, wash, α-10), TM-treated cultures (α-10, wash, TM [0.4 μg/ml]), and TM-treated cultures postincubated in standard medium for 24 h (TM [0.4 μg/ml], wash, α-10). Note that attachment (□) and mannose incorporation were suppressed in TM-treated cultures (hatched columns, *b*), but returned to control levels when postincubated in α-10 (hatched columns, *c*). (*B*) Particle binding was assessed in the presence of TM with or without the protease inhibitor leupeptin. Leupeptin does not diminish the inhibitory effect of TM nor alter the particle binding ability of control cells. Each value represents the mean±SE of six replicate cultures.

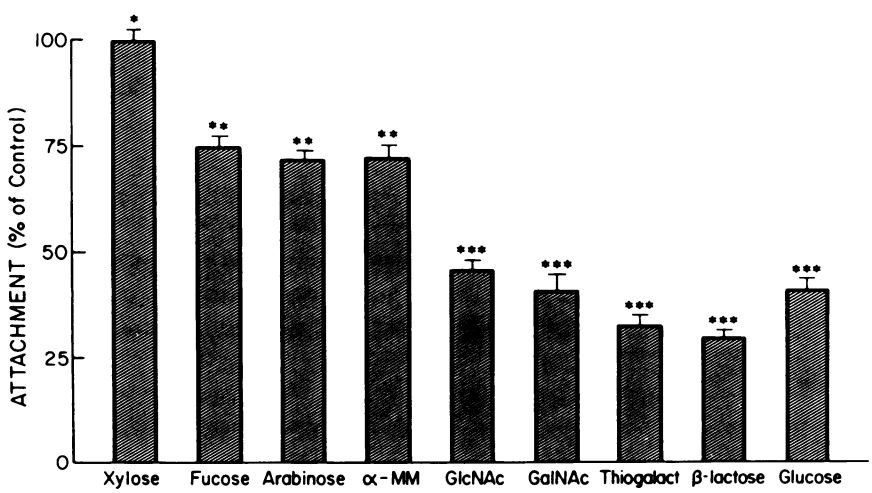

FIGURE 7 Binding of radiolabeled particles in the presence and absence of competing simple carbohydrates. Carbohydrates vary markedly in their ability to inhibit binding but the most effective (thiogalactoside and β-lactose) suppress attachment by ~60–70%. All carbohydrates were present at a concentration of 0.1 M. Each bar represents the mean±SE of four replicate cultures. α-MM, α-methyl mannoside; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; Thiogalact, thiogalactoside. *NS; **$P < 0.025$; ***$P < 0.001$.

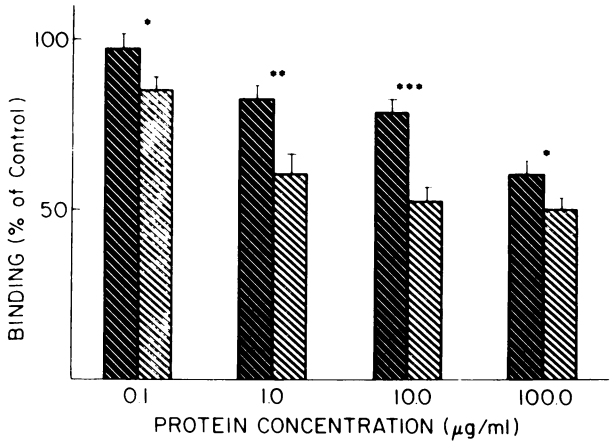

FIGURE 8 Bone particle binding to MØ in the presence of fetuin and asialofetuin. Fetuin and asialofetuin inhibit particle attachment in a dose-dependent manner. Each value represents the mean±SE of six determinations. °NS; °°$P < 0.025$; °°°$P < .010$.

a reduction in binding of 35–60%. (Parallel studies with MØ are not possible since periodate treatment markedly decreases the viability of the cells.)

DISCUSSION

Adhesiveness is a fundamental property of cells by which they interact with some of the major elements in their environment including other cells, particulate matter, and the substrate on which they reside. These adhesive interactions determine not only whether the cell may engage in phagocytic or locomotory activity, but also its ability to respond to agents in its environment (e.g., hormones) (22), its principal physiological role (e.g., the activation of the lysosomal apparatus) (23), and its phenotypic expression and proliferative functions (24, 25). In the particular instance of the MØ in bone, contact with the mineralized substrate appears essential for the development of the osteoclast phenotype (11).

In the present study, the adhesive relationships between MØ and bone have been examined, in vitro, using the elicited MØ as the paradigm for both the resorbing cell and the osteoclast precursor. The data show that MØ-bone attachment occurs rapidly in culture, yields highly reproducible binding kinetics, is temperature dependent, saturable, and, as evidenced by competition experiments between labeled and unlabeled bone, "specific." In addition, the data show

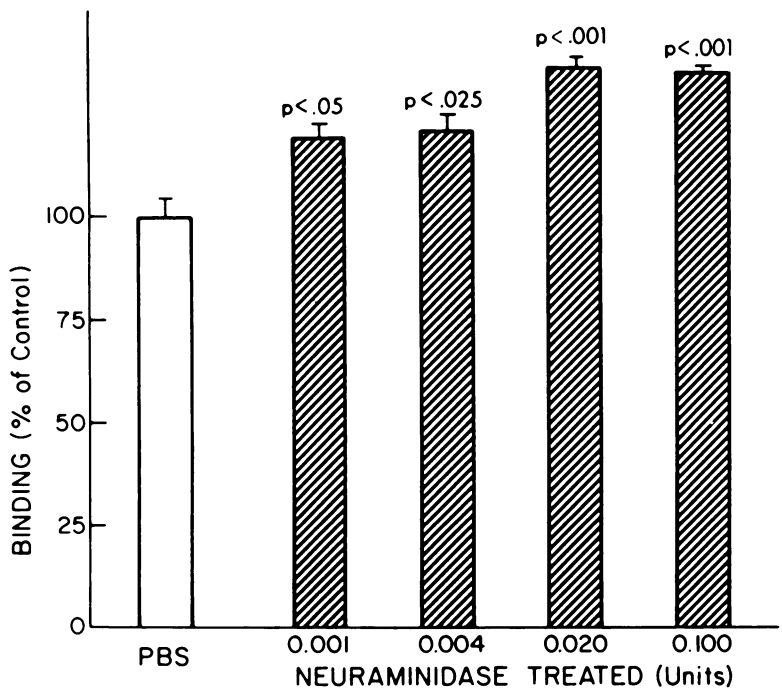

FIGURE 9 Effect of neuraminidase treatment on MØ-bone particle binding. The cells were preincubated for 30 min at 37°C in the indicated concentrations of enzyme in PBS, washed three times and assayed for particle attachment (1 h, 37°C). Neuraminidase pretreatment significantly increases particle attachment by ~20–25% ($P < 0.01$–0.001). Each value represents the mean±SE for six cultures.

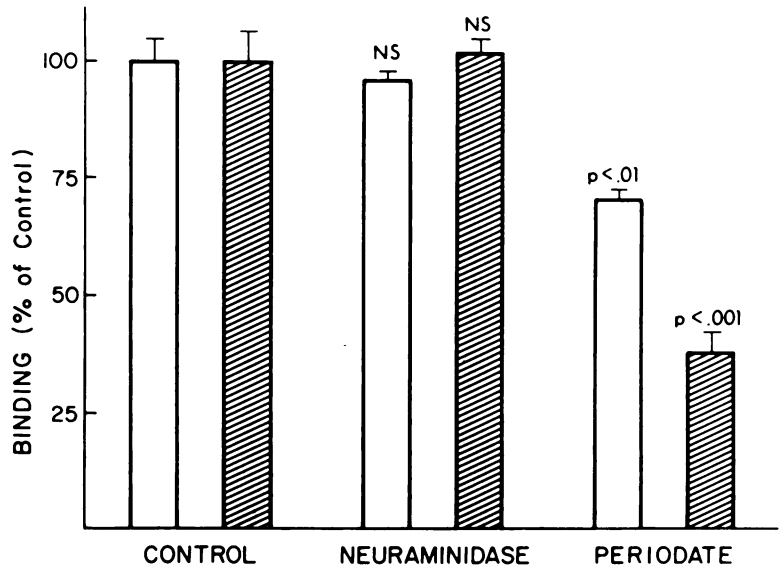

FIGURE 10 Effect of neuraminidase and periodate treatment of bone on MØ binding. Bone particles and calvaria (1 mg/ml PBS) were incubated in either 0.1 U/ml neuraminidase or 0.5 mM of $NaIO_4$ for 30 min at 37°C. The bone was washed three times in PBS and used in binding assays as previously described (1-h incubation for particles; 30-min incubation for calvaria). Periodate but not neuraminidase significantly reduces the binding affinity of bone surfaces. Each value represents the mean±SE of six determinations. Particle binding (□); calvarial binding (■).

that while MØ binding to devitalized bone particles or freshly dissected calvaria is quantitatively and, presumably, qualitatively similar, it differs fundamentally, from attachment to plastic (c.f., the carbohydrate competition experiments).[2]

Since a number of MØ surface receptor functions are mediated by carbohydrates (e.g., the uptake of lysosomal enzymes [26] and attachment of unopsonized bacteria [27]), we wondered whether these compounds might also play a role in MØ-bone attachment. To test this possibility, four different experimental strategies were used: (*a*) preincubation of MØ with the glycosylation inhibitors TM and SW; (*b*) use of simple sugars and fetuin as competitive inhibitors of MØ-bone attachment; (*c*) pretreatment of cells and bone with neuraminidase; and (*d*) oxidation of bone carbohydrates with periodate.

Preincubation of MØ with TM at a concentration sufficient to significantly inhibit glycosylation ([^{3}H]mannose incorporation), but not protein synthesis or cell survival, results in a corresponding diminution in cell-bone attachment. These inhibitory effects are reversible, demonstrating that the metabolism of the cell is not permanently altered and can take place in the presence of the protease inhibitor, leupeptin. The finding that the TM-induced effects occur in the presence of leupeptin suggests that the decreased binding is due directly to the loss of some essential carbohydrate moieties on the cell surface and not to enhanced membrane proteolysis (16).

A similar conclusion follows from the results obtained with SW, a compound that blocks the cleavage of mannose residues from primitive oligosaccharides (17). SW-treated MØ, which should be enriched in exposed mannose groups, are strongly inhibited in cell-bone binding indicating (*a*) the likely involvement of oligosaccharides in attachment and (*b*) a minor role for mannose residues in the process. The latter conclusion is further supported by the failure of mannose, yeast mannan (data not shown), and mannose-derived bovine serum albumin to block cell-bone attachment.

The observations on the inhibitory action of simple

[2] The authors are aware that the attachment of cells to bone is a complex process probably involving multiple ligands and receptors, as well as the active participation of other components in the cell and cell membrane (c.f., the temperature dependence of binding). Thus, our measurements of "saturation" and "specificity" cannot fulfill the usual criteria for making such determinations and, therefore, cannot be used to derive such ideal information as dissociation constants (K_d) and receptor number. However, we do believe the present assays are rigorous enough to measure rates of attachment, temperature optima, and the role of agents, such as carbohydrates, in the attachment process.

carbohydrates on cell-bone attachment are also consistent with a role for saccharides in the binding process. As the data show, some carbohydrates are strongly inhibitory, while others have only moderate or no effect. Presumably, the most potent inhibitors suppress attachment because they are present in oligosaccharides or glycoproteins that link cells to bone and, therefore, compete with these complex carbohydrates for cell- or bone-associated lectins. The lack of inhibition when carbohydrates are present only before assay is consistent with this conclusion. A similar mechanism likely accounts for the suppressive effect of fetuin and its asialo-derivative on cell-bone attachment. These fetal glycoproteins are known to bind to cell surfaces via their glycosyl residues, and can be inhibited from this activity by two of the carbohydrates that block MØ attachment to bone (thiogalactoside and β-lactose [17]). Thus, when present concurrently, fetuin and bone would compete for the same cell surface lectins. This hypothesis is supported by the observation that preincubation of MØ, but not bone, with fetuin or asialofetuin leads to inhibition of attachment. The fact that asialofetuin is the more potent inhibitor is also consistent with the notion that galactosides are critical carbohydrates in binding. The latter residues are more exposed in fetuin from which terminal sialic acid groups have been removed.

Further support for the involvement of saccharides in MØ-bone attachment is also to be found in the studies with neuraminidase and periodate. Pretreatment of cells with the enzyme leads to significant enhancement in binding activity, indicating that sialic acid residues play an important role in regulating cell-bone attachment. Since a comparable increase is not achieved when bone is similarly pretreated, this observation suggests that the "regulatory" sialic acid moieties are located exclusively on the cell surface. On the other hand, the reduction in binding observed following the treatment of bone with the oxidizing agent, periodate, indicates that matrix-associated carbohydrates are also involved in the binding process.

However, it is possible that the effects of TM, SW, and neuraminidase treatment on the MØ alter the cell surface expression and/or biological activity of some cell surface glycoconjugate that does not function simply as a lectin receptor.

We noted above that two important MØ activities in bone, osteoclast differentiation and resorption, appear dependent upon the establishment of intimate physical contact between the precursor/resorptive cell and the bone surface. The data from the present study indicate that this attachment is mediated by saccharides and is, therefore, similar to other binding mechanisms that have been described for MØ, e.g., the uptake of bacteria. In a companion paper (28), we show that the paucity of osteoclasts present in osteomalacia may reflect defective binding of precursor cells to bone due to altered matrix oligosaccharides.

ACKNOWLEDGMENT

This work was supported by National Institutes of Health grants DE-05413 and DE-04629.

REFERENCES

1. Weisbrode, S. E., C. C. Capen, and L. A. Nagode. 1973. Fine structural and enzymatic evaluation of bone in thyroparathyroidectomized rats receiving various levels of vitamin D. *Lab. Invest.* 28:29–37.
2. Vaes, G. 1980. Collagenase, lysosomes, and osteoclastic resorption. *In* Collagenase in Normal and Pathological Connective Tissues. D. E. Woolley and J. M. Evanson, editors. John Wiley & Sons, Inc., New York. 185–207.
3. Teitelbaum, S. L., C. C Stewart, and A. J. Kahn. 1979. Rodent peritoneal macrophages as bone-resorbing cells. *Calcif. Tissue Int.* 27:255–261.
4. van Furth, R. 1982. Current view on the mononuclear phagocyte system. *Immunobiology.* 161:178–185.
5. Burger, E. H., J. W. M. van der Meer, J. S. van de Gevel, J. C. Gribnau, C. Wil Thesingh, and R. van Furth. 1982. In vitro formation of osteoclasts from long-term cultures of bone marrow mononuclear phagocytes. *J. Exp. Med.* 156:1604–1614.
6. Ko, J. S., and G. W. Bernard. 1981. Osteoclast formation *in vitro* from bone marrow mononuclear cells in osteoclast-free bone. *Am. J. Anat.* 161:415–425.
7. Kimmel, D. B., and W. S. S. Jee. 1980. Bone cell kinetics during longitudinal bone growth in the rat. *Calcif. Tissue Int.* 32:123–133.
8. Kahn, A. J., C. C. Stewart, and S. L. Teitelbaum. 1978. Contact-mediated bone resorption by human monocytes *in vitro*. *Science (Wash. DC).* 199:988–990.
9. Blair, H., A. J. Kahn, J. Sadow, J. D. Malone, and S. L. Teitelbaum. 1981. *Biochem. Biophys. Res. Commun.* 100:959–964.
10. Kahn, A. J., P. A. Seyer, and M. D. Krukowski. 1982. Recruitment of osteoclast precursors is coupled to osteoclastic activity. *J. Dent. Res.* 62:256.
11. Krukowski, M., and A. J. Kahn. 1982. Inductive specificity of mineralized bone matrix in ectopic osteoclast differentiation. *Calcif. Tissue Res.* 34:474–479.
12. Schenk, R. K., D. Spiro, and J. Wiener. 1967. Cartilage resorption in the tibial epiphyseal plate on growing rats. *J. Cell Biol.* 34:275–291.
13. Irving, J. T., and C. S. Handelman. 1963. Bone destruction by multinucleated giant cells. *Am. Assoc. Adv. Sci. Publ.* 75:515–530.
14. Goldman, R., and Z. Bar-Shavit. 1979. A dual effect of normal and stimulated macrophages and their conditioned media on target cell proliferation. *J. Natl. Cancer Inst.* 63:1009–1010.
15. Keefer, L. M., and P. De Meyts. 1981. Glycosylation of cell surface receptors: tunicamycin treatment decreases insulin and growth hormone binding to different levels in cultured lymphocytes. *Biochem. Biophys. Res. Commun.* 101:22–29.
16. Olden, K., R. M. Pratt, and K. M. Yamada. 1979. Role of carbohydrate in biological function of the adhesive glycoprotein fibronectin. *Proc. Natl. Acad. Sci. USA.* 76:3343–3347.

17. Dorling, P. R., C. R. Huxtable, and S. M. Colegate. 1980. Inhibition of lysosomal α-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*. *Biochem. J.* 191:649–651.
18. Raz, A., and R. Lotan. 1981. Lectin-like activities associated with human and murine neoplastic cells. *Cancer Res.* 41:3642–3647.
19. Marikovsky, Y., R. Lotan, H. Lis, N. Sharon, and D. Danon. 1976. Agglutination and labeling density of soybean agglutinin on young and old human red blood cells. *Exp. Cell Res.* 99:453–456.
20. Czop, J. K., D. T. Fearon, and F. K. Austen. 1978. Membrane sialic acid on target particles modulates their phagocytoses by a trypsin-sensitive mechanism on human monocytes. *Proc. Natl. Acad. Sci. USA.* 75:3831–3835.
21. Spiro, R. G., and V. S. Bhoyroo. 1974. Structure of the *O*-glycosidically linked carbohydrate units of fetuin. *J. Biol. Chem.* 249:5704–5717.
22. Gospodarowicz, D., and D. K. Fujii. 1981. The extracellular matrix and the control of cell proliferation and differentiation. *In* Cellular Responses to Molecular Modulators. L. W. Mozes, J. Schultz, W. A. Scott, and R. Werner, editors. Academic Press, Inc., New York. 113–132.
23. Morland, B. 1979. Studies on selective induction of lysosomal enzyme activities in mouse peritoneal macrophages. *J. Retic. Endothel. Soc.* 26:749–762.
24. Folkman, J., and A. Moscona. 1978. Role of cell shape in growth control. *Nature (Lond.).* 273:345–349.
25. Glaser, L. 1980. From cell adhesion to growth control. *In* The Cell Surface: Mediator of Developmental Processes. S. Subtelny and N. K. Wessells, editors. Academic Press, Inc., New York. 79–97.
26. Sly, W. S. 1980. Saccharide traffic signals in receptor-mediated endocytosis and transport of acid hydrolases. *In* Structure and Function of Gangliosides. L. Svennerholm, P. Mandel, H. Dreyfus, and P.-F. Urban, editors. Plenum Publishing Corp., New York. 433–451.
27. Bar-Shavit, Z., R. Goldman, I. Ofek, N. Sharon, and D. Mirelman. 1980. Mannose-binding activity of *Escherichia coli*: a determinant of attachment and ingestion of the bacteria by macrophages. *Infect. Immun.* 29:417–424.
28. Bar-Shavit, Z., A. J. Kahn, and S. L. Teitelbaum. 1982. Defective binding of macrophages to bone in rodent osteomalacia and vitamin D deficiency. In vitro evidence for a cellular defect and altered saccharides in the bone matrix. *J. Clin. Invest.* 72:526–534.