an effector-to-target ratio of 100:1) (*24*). Again, previous sensitization of F_1 animals with lymphocytes from one of the two parental strains reduced the NK-mediated lysis of T cell blasts.

Second, in some instances heterozygotes might be reactive to homozygotes. For example, heterozygotes that bear HLA-Cw14 may behave as homozygotes because of low or no expression of the HLA-Cw14 allele. Thus, they might develop NK cells that lyse NK-1^+ (but not NK-2^+) homozygous cells, paralleling at least in part the situation observed in murine hybrid resistance. Both of these interpretations would make the present observations consistent with the view that human NK alloreactivity and murine hybrid resistance are analogous. In any event, the phenomena described here are important in understanding the biology of NK recognition and may have a bearing on the matching of bone marrow donors and recipients in allogeneic bone marrow transplantation in humans.

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10. A 338-bp fragment spanning the first and second exons of HLA-C was selectively amplified from genomic DNAs by the polymerase chain reaction (PCR). The primers used for amplification were CCCGAACCCTC(C/A)TCCTGCT (5′, nucleotides 17 to 35) and ACGGCGCCCGCGGCTCCC (3′, nucleotides 206 to 223). Reaction conditions for the amplification were as described (*6*). Amplified fragments were gel-purified, subcloned into pCR1000 (Invitrogen), and sequenced by dideoxynucleotide chain termination. Out of four independent clones analyzed from each PCR product, at least one showed a sequence identical to the reported sequence for HLA-Cw14 (*7, 12*); the other clones represented the second HLA-C allele of the heterozygote.
11. For oligonucleotide typing of HLA-Cw14, HLA-C was amplified from genomic DNAs (*10*), denatured, applied to Hybond-membranes (Amersham), and hybridized with the following ^{32}P oligonucleotides: GTATTTCTCCACATCCGTGTC (nucleotides 90 to 110, specific for HLA-Cw4, HLA-C BeWo C.1, and HLA-Cw14), TTTCTCCACATCCGTGTCCCGGCCC (nucleotides 93 to 117, specific for Cw14), and CCGTGTCCTGGCCCGGCC (nucleotides 104 to 121, specific for Cw4 and HLA-C BeWo C1). Hybridization conditions were as described in Fig. 1. Membranes were washed in 3 N tetramethylammonium chloride, 50 mM tris-HCl (pH 8.0), and 2 mM EDTA for 30 min at 59°C, 65°C, and 59°C, respectively.
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Mechanotransduction Across the Cell Surface and Through the Cytoskeleton

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Mechanical stresses were applied directly to cell surface receptors with a magnetic twisting device. The extracellular matrix receptor, integrin β_1, induced focal adhesion formation and supported a force-dependent stiffening response, whereas nonadhesion receptors did not. The cytoskeletal stiffness (ratio of stress to strain) increased in direct proportion to the applied stress and required intact microtubules and intermediate filaments as well as microfilaments. Tensegrity models that incorporate mechanically interdependent struts and strings that reorient globally in response to a localized stress mimicked this response. These results suggest that integrins act as mechanoreceptors and transmit mechanical signals to the cytoskeleton. Mechanotransduction, in turn, may be mediated simultaneously at multiple locations inside the cell through force-induced rearrangements within a tensionally integrated cytoskeleton.

The process of recognizing and responding to mechanical stimuli is critical for the growth and function of living cells. Many sensory functions including touch, hearing, baroreception, proprioception, and gravity sensation involve specialized mechanotransduction mechanisms. Development of tissue pattern is also exquisitely sensitive to changes in mechanical stress (*1*). Nevertheless, the molecular mechanism by which individual cells recognize and respond to external forces is not well understood. Stretch-sensitive ion channels, adenylate cyclase, and protein kinase C change their activity in response to applied stress (*2–4*). However, these signaling pathways are likely to lie downstream from the initial mechanoreception event at the cell surface. For example, activation of these signaling molecules appears to be mediated though changes in the cytoskeleton (CSK) (*2, 4, 5*). Although changes in CSK organization are a ubiquitous response to mechanical perturbation (*4, 6, 7*), the mechanism by which forces are transmitted across the cell surface and transduced into a CSK response remains unknown.

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Analysis of mechanotransduction in specialized force-sensing cells, in both plants and animals, suggests that the cell's extracellular matrix (ECM) attachments are the sites at which forces are transmitted to cells (*6, 8*). As in any architectural structure, mechanical loads are transmitted across the cell surface and into the cell by means of structural elements that are physically interconnected. Transmembrane ECM receptors, such as members of the integrin family, are excellent candidates for mechanoreceptors because they bind actin-associated proteins within focal adhesions and thereby physically link ECM with CSK microfilaments (*9*). The possibility that ECM receptors mediate mechanotransduction is supported by the finding that stretching flexible ECM culture substrata alters CSK organization and induces biochemical changes in adherent cells (*10*). However, in these stretching studies, it is not possible to separate effects due to transmembrane force transfer from those due to global shape changes and generalized deformation of the plasma membrane and CSK.

To determine whether ECM receptors provide a specific molecular path for mechanical signal transfer to the CSK, we devised a method in which controlled mechanical loads could be applied directly to specific cell surface molecules without producing large-scale changes in cell shape (Fig. 1). We modified a cell magnetometry

system (*11*) by allowing cells to bind spherical ferromagnetic microbeads that were coated with specific receptor ligands that mediate attachment but not cell spreading (*12, 13*). By magnetizing these surface-bound beads in one direction and then applying a second, weaker magnetic field oriented at 90°, we were able to twist the beads in place and thereby exert a controlled shear stress (0 to 68 dyne/cm^2) on bound cell surface receptors. An in-line magnetometer was used to simultaneously measure changes in the orientation of the magnetized beads and hence to quantitate angular strain produced in response to the applied stress.

Adherent endothelial cells were first allowed to bind beads coated with a synthetic peptide containing the Arg-Gly-Asp (RGD) sequence that is a known ligand for fibronectin receptors, such as integrin $\beta_1\alpha_5$, which these cells express on their surface (*13*). Efficient transmembrane force transfer was observed in cells bound to RGD beads; the cells became stiffer and increased their resistance to mechanical deformation (bead twisting) at higher levels of applied stress, such that angular strain only reached a bead rotation of approximately 25° (Fig. 2). To demonstrate the specificity of transmembrane force transfer, we included a soluble synthetic peptide, Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP) (1 mg/ml), in the culture medium as a competitor (*14*). This fibronectin peptide inhibited CSK stiffening (Fig. 2), whereas a control hexapeptide with a single amino acid substitution (Gly-Arg-Gly-Glu-Ser-Pro) had no inhibitory effect. Beads coated with antibodies directed against integrin β_1 receptor subunits produced a similar stiffening response (Fig. 2). In contrast, surface-bound beads coated with nonspecific cell attachment ligands, such as acetylated–low density lipoprotein (AcLDL) (*15*) or bovine serum albumin (BSA), were not nearly as restricted in their rotation (Fig. 2).

To confirm that applied mechanical loads were indeed transmitted to the CSK, we measured the mechanical properties of cells bound to RGD beads before and after disrupting microfilament lattice integrity with a low concentration of cytochalasin D (0.1 μg/ml), which had minimal effects on cell shape. Angular strain increased after exposure to cytochalasin for only 15 min (Fig. 2). Efficient force transfer and associated CSK stiffening also correlated with focal adhesion formation, as defined by the recruitment of talin, vinculin, and α-actinin to the site of bead binding (Fig. 3). These focal adhesion proteins, which appeared along the surface of RGD beads but not AcLDL beads, form the molecular bridge that physically interlinks integrins with actin microfilaments (*9*). Recruitment of talin also appears to be required for cell spreading on ECM (*16*).

Importantly, disruption of microfilament lattice integrity with cytochalasin D did not completely suppress CSK stiffening (Fig. 4A), suggesting that other filament systems may also contribute to the CSK response to force. Disruption of microtubules or intermediate filaments with nocodazole (10 μg/ml) or acrylamide (4 mM; *17*), respectively, inhibited the stiffening response by approximately 25% (Fig. 4A), and no additive effect was observed when they were combined. Combination of cytochalasin D with acrylamide reduced stress-induced CSK stiffening by more than 85%, and combination with nocodazole resulted in complete suppression (Fig. 4A). Thus, although integrins may initially transmit forces to microfilaments within focal adhesions, higher order structural interactions among all three CSK filament systems appear to be responsible for efficient transduction of the mechanical stimulus into a cellular response. The finding that actin microfilaments contribute the most to cell stiffness is consistent with recent data which show that networks of purified actin polymers exhibit a higher shear modulus than networks containing microtubules or intermediate filaments (*18*).

How could a "solid" lattice composed of interconnected microfilaments, microtubules, and intermediate filaments (*19*) respond dynamically as a single integrated unit? Consider the observation that CSK stiffness increased in direct proportion to the stress applied to integrins (the slope of the curve in Fig. 4A is linear). This type of mechanical behavior is not commonly seen in man-made materials, but it is often observed in biological tissues (*20*). This mechanical response cannot be explained by current theories (*20*). We have proposed that in the construction of cells a building system may be used that was first described by the architect-inventor Buckminster Fuller and that depends on tensional integrity (tensegrity) rather than compressional continuity (*21*). Tensegrity "cell" models that incor-

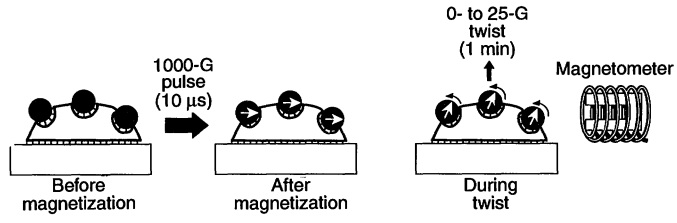

Fig. 1. The magnetic twisting device. Microbeads (5 × 10^4 in each well) were allowed to bind to cell surfaces for 10 to 15 min, and unbound beads were removed before magnetic manipulation was initiated. Brief application of a strong external magnetic field (1000 G for 10 μs) resulted in magnetization and alignment of the magnetic moments of all surface-bound beads. We then applied defined mechanical stresses (0 to 68 dyne/cm^2) without remagnetizing the beads, using a weaker "twisting" magnetic field (0 to 25 G) applied perpendicular to the original field. We measured the average bead rotation (angular strain) induced by the twisting field by using a magnetometer to measure changes in the component of the remanent magnetic field vector in the direction of the original magnetization as a function of time (*11*). In the absence of force transmission across the cell surface, the spherical beads would twist in place by 90° into complete alignment with the twisting field, and the remanent field vector would immediately drop to zero. In contrast, transmission of force to the CSK would result in increased resistance to deformation and decreased bead rotation.

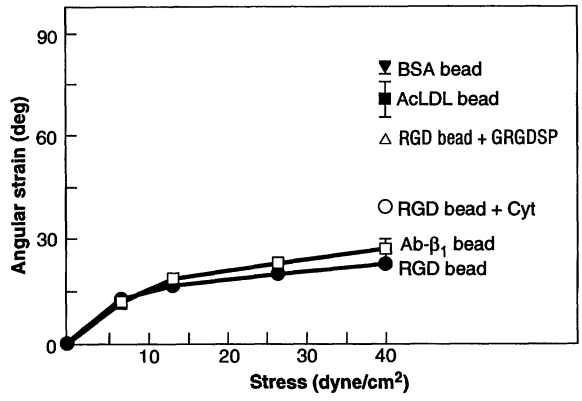

Fig. 2. Stress-strain relation measured with magnetic microbeads attached to the surfaces of living cells. Applied stress was determined by a calibration technique in which the same beads were twisted in a standard solution of known viscosity (*22*). Angular strain (bead rotation) was calculated as the arc cosine of the ratio of remanent field after 1 min of twist to the field at time 0. Angular strain is plotted here as degrees. Bead coatings were as follows: RGD, Arg-Gly-Asp–containing synthetic peptide; Ab-β_1, antibodies against integrin β_1; AcLDL, acetylated–low density lipoprotein; BSA, bovine serum albumin; GRGDSP, soluble fibronectin peptide (1 mg/ml added for 10 min); Cyt, cytochalasin D (0.1 μg/ml). Measurements analyzing the effects of different bead coatings with or without GRGDSP were made at stresses from 0 to 40 dyne/cm^2; for clarity, intermediate data points are shown only for Ab-β_1 and RGD beads that exhibit integrin-dependent stiffening. The effects of cytochalasin D were measured only at the highest stress. Error bars = SEM.

porate isolated rigid struts interconnected by a continuous series of elastic tensile threads predict cell shape changes and mimic specific structural patterns that are observed within the CSK of living cells (*21*).

To explore whether cells might use tensegrity to mediate mechanotransduction within the CSK, we carried out stress-strain measurements with a stick and elastic string tensegrity model. When increasing force (metal weights) was applied to these models, the mechanically interdependent structural elements rearranged without topological disruption or loss of tensional continuity (Fig. 4B). A plot of stiffness versus applied stress (force) based on these models (Fig. 4C) mimicked the linear response exhibited by the CSK of living cells (Fig. 4A) as well as by intact biological tissues (*20*). This linear response was in direct contrast to the behavior exhibited by nonprestressed tensile filaments taken from the same structure (Fig. 4C). Stiffness of the compression-resistant struts was essentially infinite over the range of forces applied. Viewed in this light, the CSK response to applied stress appears to be a property of the integrated system and not a characteristic of any one of its individual parts. Gels containing purified CSK filaments (for example, F-actin) that lack structural continuity and internal tension (prestress) either do not exhibit force-induced stiffening or, if they do, the response is nonlinear (*18, 22*) and appears similar to that exhibited by a non-prestressed tensile filament (Fig. 4C).

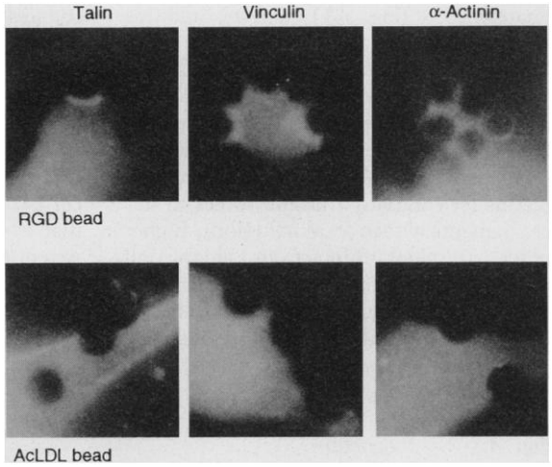

Fig. 3. Immunofluorescence micrographs of cells bound to beads coated with RGD or AcLDL and immunostained for the focal adhesion proteins talin, vinculin, and α-actinin.

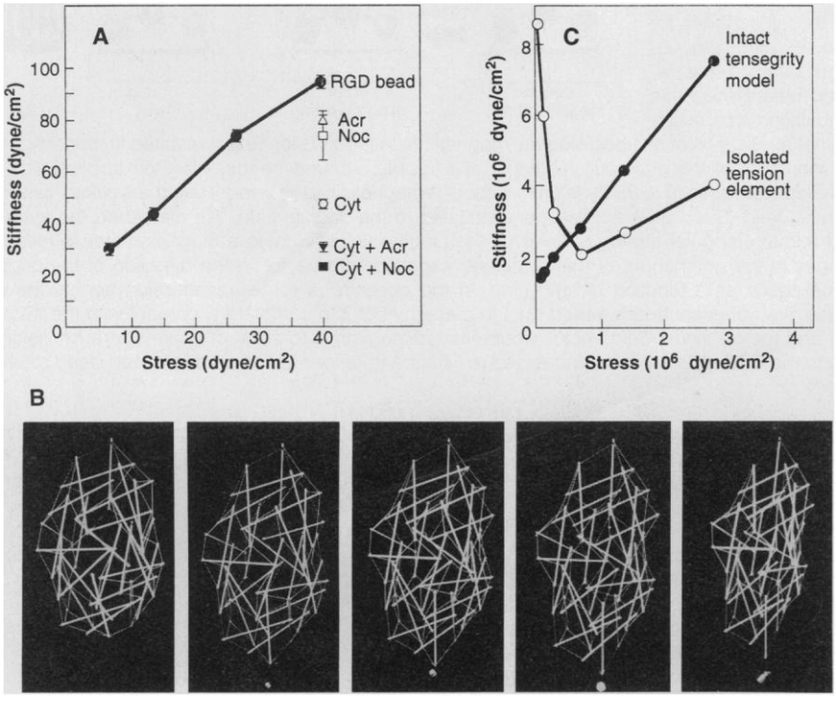

Fig. 4. Continuum mechanics analysis of living cells and a three-dimensional tensegrity model. (**A**) Stiffness of the CSK of living cells was defined as the ratio of stress to strain (in radians) at 1 min of twisting. Noc, nocodazole (10 μg/ml); Acr, acrylamide (4 mM); Cyt, cytochalasin D (0.1 μg/ml). (**B**) A tensegrity cell model under different mechanical loads. This model consisted of a geodesic spherical array of wood dowels (0.3 cm by 15 cm) and thin elastic threads (0.06 cm by 6 cm). The model was suspended from above and loaded, from left to right, with 0-, 20-, 50-, 100-, or 200-g weights on a single strut at its lower end. (**C**) Stiffness of the stick and string tensegrity model was defined as the ratio of applied stress to strain (linear deformation of the entire structure). Similar measurements were carried out with an isolated tension element, that is, a single thin elastic thread of a size similar to that found in the model.

Thus, our experimental data are consistent with the possibility that the CSK is organized as a tensegrity network. In living cells, contractile microfilaments generate and distribute tension to all CSK filament systems (*23*). In addition, microfilaments resist compression locally when either cross-linked within large bundles or contracted to their shortest length (*21*). Microtubules also resist compression in cells (*21, 24*), possibly because they are stabilized against buckling by lateral interconnections with tensionally stiffened intermediate filaments (*25*). Our finding that a combination of acrylamide and nocodazole did not further reduce CSK stiffness supports this possibility that intermediate filaments and microtubules resist compression as a paired unit. The tensegrity paradigm therefore provides a novel mechanism for CSK integration (*21*) as well as a plausible explanation for why the CSK stiffening response is linear in cells (Fig. 4A) and tissues (*20*). It also could explain how a local stress, induced by ligation of a subset of CSK-associated membrane receptors, can result in global modulation (immobilization) of receptors over the entire cell surface (*26*).

On a more general level, our findings suggest that the balance of mechanical forces that preexists within the CSK before an external mechanical load is applied (that is, prestress) may be a critical determinant of the subsequent cellular response. This result may have direct implications for understanding specialized mechanosensory mechanisms (*6*) as well as coupling between cell shape and function (*27*). For example, the change in the level of CSK prestress that accompanies changes in cell shape may provide regulatory information to the cell (*21, 27*). Prestress of the CSK also may play a critical role in the cellular mechanism of aging, given that the load-bearing properties of any structural support element would be expected to weaken over time, if continually stressed.

Taken together, these results indicate

that at least one type of transmembrane ECM receptor, integrin β_1, can act as a mechanoreceptor in that it can transfer mechanical signals to the CSK by way of a specific molecular pathway. A cell's sensitivity to a mechanical stimulus therefore may be altered by changing ECM receptor number, location, or adhesion strength or by modulating focal adhesion formation. Other types of transmembrane molecules that interconnect with CSK filaments (for example, different integrin subunits, cadherins, or cell surface proteoglycans) may also transfer external mechanical signals to the CSK. The magnetic twisting device provides a simple method to directly address this possibility. In addition, these results suggest that transfer of force from integrins to the CSK may represent a proximal step in an intracellular mechanical signaling cascade that leads to global CSK rearrangements and simultaneous mechanotransduction events at multiple locations inside the cell (*21*, *28*). If cells use a tensegrity-based transduction system, then mechanical signal transfer throughout the entire cell would be essentially instantaneous and thus more rapid than any diffusion-based signaling system.

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12. Capillary endothelial cells were plated (3×10^4 cells per well) on fibronectin-coated bacteriological plastic (96-well Removawells, Immunolon II) and cultured for 6 to 10 hours in chemically defined medium (*27*) before bead addition. Spherical ferromagnetic beads (5.5 μm in diameter, 1 mg/ml) [W. Moller, C. Roth, W. Stahlhofen, *J. Aerosol Sci.* **21**, S657 (1990)] were coated with RGD-containing peptide (Peptite 2000), AcLDL, BSA, or anti-integrin β_1 antibodies as previously described (all at 50 μg/ml) (*13*).
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Expression of *Pseudomonas aeruginosa* Virulence Genes Requires Cell-to-Cell Communication

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Pseudomonas aeruginosa **is an opportunistic human pathogen that causes a variety of infections in immunocompromised hosts and individuals with cystic fibrosis. Expression of elastase, one of the virulence factors produced by this organism, requires the transcriptional activator LasR. Experiments with gene fusions show that gene *lasI* is essential for high expression of elastase. The *lasI* gene is involved in the synthesis of a diffusible molecule termed *Pseudomonas* autoinducer (PAI). PAI provides *P. aeruginosa* with a means of cell-to-cell communication that is required for the expression of virulence genes and may provide a target for therapeutic approaches.**

The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen capable of secreting many extracellular virulence factors. Among these are the products of the *aprA* gene, which encodes alkaline protease (*1*), the *toxA* gene encoding toxin A (*2*, *3*), and the products of the *lasA* and *lasB* genes, each of which encodes a protease with elastolytic activity (*4*, *5*). The role of elastase as a virulence factor is supported by the list of substrates that it uses, including elastin (*1*), human immunoglobulins G and A (*6*, *7*), some collagens (*8*), serum α_1-proteinase inhibitor (*9*), and components of the complement system (*10*).

The product of the *lasR* gene (LasR) has been shown to be required for the transcrip-

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