The prototypic immunoadhesin is an antibody-like molecule that fuses the Fc region of an immunoglobulin and the ligand-binding region of a receptor or adhesion molecule. In this article, we review some important structural and functional principles of immunoadhesins. In addition, we highlight some unique advantages of immunoadhesins as experimental tools in biology, as well as some of their exciting potential applications in medicine.

Monoclonal antibodies (mAbs) have proved to be extremely useful not only as research tools, but also as diagnostic tools and therapeutic agents. Human mAbs are better suited to therapeutic use in humans because they are much less immunogenic than mAbs derived from nonhuman species. However, it has been difficult to obtain human mAbs, especially those directed at human antigens, for at least two reasons. First, humans are generally tolerant to their own antigens. Second, ethical considerations place restrictions on the active immunization of humans for the purpose of generating mAbs. Several strategies each exploiting the power and versatility of genetic engineering – have been pursued to circumvent the difficulty in obtaining human mAbs. One approach is to minimize the amount of nonhuman sequences by combining framework sequences from a human mAb with antigen-binding sequences from a nonhuman mAb, for example, in chimeric or humanized antibodies. Another strategy is to select antibodies from bacteriophage expression libraries encoding the human antibody gene repertoire. A third method is to generate human antibodies in animals such as mice by replacing the genetic loci for endogenous antibodies with gene elements for human antibodies. A fourth strategy, which is the subject of this review, is to generate an antibody-like molecule by combining framework sequences from a human mAb with sequences from a human protein that carries a target-recognition function.

The most common example of this type of fusion protein combines the hinge and Fc regions of an IgG1 heavy chain with the extracellular domain (ECD) of a type I transmembrane protein, usually a receptor or an adhesion molecule (Box 1). This type of molecule is called an ‘immunoadhesin’, because it combines ‘immune’ and ‘adhesion’ functions. (Other frequently used names are ‘Ig-chimera’, ‘IgG’ or ‘Fc-fusion protein’, or ‘receptor-globulin’.) In this review, we summarize the many examples of immunoadhesins in the literature, and discuss some essential structural and functional features of this expanding family of recombinant proteins. In addition, we describe a range of applications for immunoadhesins as research tools and as potential human therapeutic agents.

Immunoadhesin structure

The majority of immunoadhesins combine the hinge and Fc regions of an IgG1 heavy chain with the extracellular domain (ECD) of a type I transmembrane protein, usually a receptor or an adhesion molecule (Fig. 1). The same basic principle has been applied successfully to other types of fusion partners, including cytokines, growth factors and enzymes. To date, more than fifty immunoadhesins have been reported (Table 1). The prototypic immunoadhesin is a disulfide-linked homodimer, which resembles an IgG1 molecule, but which lacks C1 domains and light chains. The dimeric structure of the expressed and purified molecule can be confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), in the presence of a reducing agent to separate the polypeptide chains (Fig. 2a). In addition, functional domains can be identified by immunoblotting (Fig. 2b) and ligand blotting (Fig. 2c). In the absence of light chains, deletion of the C1 domain improves assembly and secretion of immunoadhesin by the host cell. Placing the fusion junction at the flexible hinge facilitates proper folding of domains and helps to preserve the functions of both parts of the molecule.

Immunoadhesins can be expressed efficiently in a variety of host cells, including myeloma cell lines, Chinese hamster ovary cells, monkey COS cells, human embryonic kidney 293 cells and baculovirus-infected insect cells. In these systems, the immunoadhesin polypeptides are assembled and secreted into the cell culture medium. The molecule can then be purified substantially by protein A or protein G affinity chromatography, in much the same manner as...
An antibody is a Y-shaped molecule, composed of two identical light chains (grey) and two identical heavy chains (white). Both light and heavy chains comprise variable and constant regions. The four chains are held together by disulfide bonds (dotted lines), which are located in a flexible region of the heavy chain, known as the hinge region (tinted). Variable regions (hatched) of both light and heavy chains combine to form two identical antigen-binding sites, one on each arm of the Y. Heavy-chain constant regions define five classes of antibodies (IgA, IgD, IgE, IgG, and IgM), each with its own class of heavy chain—\( \alpha, \delta, \epsilon, \gamma, \) and \( \mu \), respectively. Each antibody class (termed an 'isotype') has distinct structural characteristics (e.g., IgG differs somewhat from other isotypes in Fc structure), and each has different biological properties. With isotypes such as IgM and IgA, multimeric assemblies of four-chain units produce antibody molecules with ten and four antigen-binding sites, respectively.

In addition, there are a number of subclasses of IgG and IgA immunoglobulins; for example, within the human IgG isotype, there are four subclasses (IgG1, IgG2, IgG3 and IgG4) having \( \gamma_{1}, \gamma_{2}, \gamma_{3}, \) and \( \gamma_{4} \) heavy chains, respectively (the illustrated structure is that of a human IgG1 antibody). Effector functions of antibodies, such as complement activation, binding to phagocyte-Fc receptors, antigen-dependent cellular cytotoxicity, and transport across the placenta, are mediated by structural determinants within the Fc region of the heavy chains.

In a typical immunoadhesin, the variable regions of the antibody molecule, which are responsible for antigen recognition, are replaced by the ligand-binding region of a receptor, while the antibody Fc region is retained. Depending on the Ig isotype, the Fc region can confer a long half-life in the circulatory system, as well as confer immune effector functions. In addition, the hinge region is retained to provide conformational flexibility that can allow the Fc and receptor regions to function independently. Immunoadhesins of IgG, IgM and IgE isotypes have been described.

**Box 1. Structure and composition of antibodies**

![Schematic structure of an immunoadhesin](image)

**Figure 1**

Schematic structure of a prototypic immunoadhesin. An immunoadhesin is usually derived from the parental ligand-binding protein, in this case a type I transmembrane receptor, and an IgG1 heavy chain molecule. ECD, TM and CYT refer to the extracellular, transmembrane and cytoplasmic domains, respectively, of the receptor. The variable (V\( _{H} \)) and constant (C\( _{H,1} \), hinge, C\( _{H,2} \) and C\( _{H,3} \)) regions of IgG1 heavy chain are shown. Shading has been used to distinguish regions of the two parent molecules that are used to form the immunoadhesin. The N-linked carbohydrate chain in C\( _{H,2} \) is indicated by a closed square. The hinge-region sequence indicates the location of proteolytic cleavage to generate monovalent receptor fragments. (Redrawn, with permission, from Ref. 90.)
## Table 1. Immunoadhesins

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of various Ig isotypes. When expressed in transfected cells, these fusion proteins were assembled properly and secreted. Moreover, like the soluble CD4-receptor molecule, these CD4-based immunoadhesins bound to gp120, the envelope glycoprotein of human immunodeficiency virus (HIV)-1, thus preventing the virus from infecting human T cells in culture. The ‘immunoadhesin concept’ has been expanded to fusion partners that do not belong to the Ig gene superfamily; initially, through the production of an immunoadhesin based on the L-selectin adhesion molecule and, later, through the production of immunoadhesins based on the receptor for tumor necrosis factor (TNF; Refs 28–30).

### Immunoadhesins with unique structural characteristics

Several structural variations on the basic immunoadhesin theme are possible (Fig. 3a–3f). One such variation is multimeric immunoadhesins. While an
### Immunoadhesins

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### Other cell surface proteins

| CD28, B7, B7-2            | CD28    | Studies on stimulation of T cells by B cells | 64, 73 |
| B61                       | Eck     | Role of Eck in inflammation | 65 |
| β-Neurexin                | β-Neurexin ligand | Characterization of cleaved signal sequences in β-neurexins | 66 |
| CD3, CD48                 | CD3, CD45 | Ligand identification | 67, 68 |
| CD5                       | CD5 ligand | Studies on stimulation of T cells by B cells | 64 |
| CD6                       | ALCAM   | Studies on binding activity of cloned ligand | 69 |
| CD22, CD45, other         | CD45, other sialoglycoproteins | Ligand identification; studies on the role of CD22 in T-B-cell interaction; characterization of binding determinants of sialo-oligosaccharide ligands | 70-72 |
| CD28, B7, B7-2            | C3 fragments | Studies on stimulation of T cells by B cells | 64, 73 |
| CD31, CD31                | CD31    | Identification of CD31 domains involved in homotypic binding | 74 |
| CD44                      | Hyaluronate | Screening for tissues that contain the ligand by histochemical staining; characterization of ligand structural determinants | 75 |
| Complement R-2 (CD21)     | C3 fragments | Prevention of antibody responses to immunosuppressive and cancer therapeutic agents | 76 |
| CTLA-4                    | B7      | Identification of CTLA-4 as a second receptor for B7 | 77 |
| IgF R                     | IgF     | Inhibition of mast-cell binding of IgE as a treatment for allergic disease | 78 |
| Lysosomal membrane gp-1   | LAMP-1 ligand | Mapping epitopes of anti-ligand antibodies | 79 |
| α2-Macroglobulin receptor-associated protein | gp330 | Tissue localization of ligand by histochemical staining | 80 |
| Natriuretic peptide R     | Natriuretic peptide | Mapping epitopes of anti-ligand antibodies; production of recombinant receptor for structural studies | 81, 82 |

### Soluble ligands

| IL-2, IL-10, Her4g          | IL-2R, IL-10R, Her4/p180m84 | Extension of the half-life of IL-2 in circulation; treatment of septic shock and transplant rejection; extension of the half-life of IL-10 in circulation | 83, 84 |
| Keratinocyte growth factor | Keratinocyte growth factor receptor | Studies on Her4 signalling; receptor localization by histochemical staining | 85, 86 |

IgG-based immunoadhesin (Fig. 3a-3d) contains two binding sites for its target, an IgM-based immunoadhesin contains ten such sites (Fig. 3c). Multimeric immunoadhesins can bind their respective targets with greater avidity than their IgG-based counterparts; examples include CD4-IgM (Ref. 6), ICAM-1-IgM (Ref. 21) and CD2-IgM (Ref. 67). A second structural variant is represented by immunoadhesins that contain an Fc region with unique effector functions. The Fc region in IgG-based immunoadhesins can retain many functions of the parental isotype, including binding to Fc receptors and complement activation. A unique case is IgE (Fig. 3f). When binding to allergens, IgE binds via its Fc to specific high-affinity receptors on the surface of basophils and mast cells, subsequently activating degranulation. This effector function can be redirected against specific targets, by incorporating the IgE-Fc into an immunoadhesin. Thus, a CD4-IgE immunoadhesin redirects basophils to attack HIV-infected cells in culture.

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A third structural variant is exemplified by an immunoadhesin based on the hepatocyte growth factor (HGF) receptor. In this case, proteolytic processing of the receptor region of the immunoadhesin results in a complex subunit structure (Fig. 3b). Each of the HGF-receptor chains in the molecule is cleaved into two subunits, which are held together by disulfide bonds. The mature HGF-receptor immunoadhesin binds to HGF and serves as a model for studying the natural processing of the endogenous receptor.

A fourth variant is an immunoadhesin that was used to produce a homodimeric cell-surface receptor (Fig. 3b). CD28, a surface protein of T cells, forms a covalent homodimer that binds to its ligand, B7, on B cells. In order to produce soluble CD28 homodimer, Linsley et al. constructed a CD28 immunoadhesin in which the only point of interchain attachment was a disulfide bond between the two CD28 domains. In this construct, the hinge Cys residues were replaced by Ser, eliminating disulfide attachment of the heavy chains.

A fifth variation is the production of bispecific immunoadhesins (Fig. 3c). This can be achieved by co-transfecting plasmids encoding two different immunoadhesins into a host cell. The co-transfected cells produce a mixture of three dimers: the two monospecific homodimers and a bispecific heterodimer, for example, E-selectin–IgG and P-selectin–IgG (Ref. 88). Heterodimer formation can be optimized by manipulating the concentration of each transfected plasmid in the reaction mix. Similar approaches have been used to generate bispecific immunoadhesins based on LFA-1 and VLA-4 (Ref. 89), as well as CD4 and L-selectin (Ref. 90).

A further extension of the bispecific immunoadhesin design is the immunoadhesin–mAb hybrid molecule (Fig. 3d). The human CD4–IgG X humanized anti-CD3 immunoadhesin–mAb hybrid is a recent example of a bispecific immunoadhesin–antibody (BIA) that comprises an immunoadhesin chain, and an antibody heavy and light chain pair. This molecule simultaneously binds to HIV gp120 on HIV-infected cells via its CD4 moiety, and to the CD3 chain of TCR on cytotoxic T lymphocytes (CTL) via its anti-CD3 moiety. The juxtaposition of the two cell types by the bispecific molecule enables the CTL to kill the HIV-infected cells selectively in vitro.

The production of bispecific immunoadhesins or immunoadhesin–mAb hybrids is generally more efficient than bispecific mAb production. This is because the number of possible subunit combinations in the former case is limited to three, compared with ten in the latter case. Consequently, host cells generate more bispecific immunoadhesin or immunoadhesin–mAb hybrid than bispecific mAb per gram of recombinant protein produced; moreover, the desired bispecific molecule can be purified more easily in the former case because the mixture of possible recombinant products is less complex.

The last variation that we discuss is the generation of cleavable immunoadhesins. Much like antibodies, immunoadhesins can be cleaved enzymatically at the hinge to release the fusion partner as a soluble protein. If the immunoadhesin is purified prior to cleavage, for example by protein A chromatography, and if the cleaving protease is immobilized, then the released fusion partner can be obtained in pure form, simply by removing its accompanying Fc fragment with protein A. Thus, papain digestion has proved to be useful for generating soluble receptors from CD4–IgG (Ref. 91), native peptide receptor C–IgG (Ref. 82) and E-selectin–IgG (Ref. 18). However, in other cases, the fusion partner itself may contain sequences that are susceptible to cleavage by papain (which is a relatively promiscuous protease). To circumvent this problem, sequences that are recognized by more-selective proteases, such as thrombin, have been introduced into the hinge region of some immunoadhesins (Ref. 80,85). A further development of this concept is the incorporation of a short sequence, which is recognized with exquisite specificity by an engineered subtilisin mutant that cleaves peptide bonds by substrate assisted catalysis, into the immunoadhesin hinge (Ref. 80,82).

**Immunoadhesins as research tools**

Because it is generally straightforward to construct immunoadhesins, to express them in mammalian cells, and to purify them, they have been used frequently as research reagents (Table 1). Below, we highlight a few experimental areas in which immunoadhesins have been particularly useful; for additional examples, see earlier reviews (Refs. 90,91).
Study of the biological function or the mode of action of receptors and ligands

Immunoadhesins can be used as antagonists or as agonists that block or mimic physiological molecular interactions. As such, immunoadhesins can be used to investigate the biological role and the mechanism of action of the parent molecules from which they were derived. For example, ICAM-2-IgG has been used to show that ICAM-2 provides an important co-stimulatory signal during TCR-mediated T-cell activation, by interacting with a counter receptor, CD11a/CD18 (Ref. 23). Similarly, a Fas receptor-based immunoadhesin has helped to establish an essential role for Fas ligand in the killing of targets by CTL (Ref. 38).

Chan and Aruffo\(^2\) explored the role of the β1 integrin VLA-4 in mediating lymphocyte transendothelial migration using immobilized VCAM-1-IgG as a target ligand. An LFA3 immunoadhesin was used to investigate the involvement of phospholipase Cγ1 in CD2-mediated signal transduction in T cells\(^2\). In another study, a B61 immunoadhesin was used to establish that B61 induces angiogenesis via its receptor tyrosine kinase (Eck) during inflammation in vivo\(^5\). Lastly, immunoadhesins based on the α and β chains of the interferon (IFN)-γ receptor have helped to demonstrate the formation of a ternary complex that contains the homodimeric ligand and two molecules of each receptor chain\(^4\). Thus, immunoadhesins can be used to probe a wide range of biological functions and, in some cases, can be used to identify the mechanisms that underlie a given function.

Identification of ligand-binding determinants

The hinge and Fc regions of an immunoadhesin form a convenient scaffold for presenting functional domains from proteins such as receptors and cell-adhesion molecules. Usually, the structural and biochemical properties of the fusion partner are retained in the immunoadhesin, perhaps as a result of the spatial separation from Fc, combined with the flexibility of the hinge. Hence, immunoadhesins have been useful for probing structure–function aspects of protein–protein interactions. In such studies, site-directed mutagenesis approaches, such as the deletion of domains and subdomains, or the substitution of individual amino acids, have been combined with immunoadhesin-based presentation. For example, a deletion series based on CD31 was studied in immunoadhesin format to identify which of the six Ig-like domains of CD31 mediate homotypic adhesion\(^7\). Similar studies have investigated the ligand binding determinants of the receptors for platelet-derived growth factor\(^2\), interleukin (IL)-4 (Ref. 51), ICAM-3 (Ref. 24), HGF (Ref. 61) and TNF (Ref. 31). The formation of a ternary complex involving gp130, IL-6 and IL-6 receptor has been investigated using domain–deletion mutants of gp130 as immunoadhesins\(^8\). In addition, individual residues of CD4 that bind to HIV-gp120 have been identified by alanine-scanning mutagenesis of CD4 as an immunoadhesin\(^9\). With a somewhat different goal, Fibi et al.\(^5\) used a human erythropoietin receptor-based immunoadhesin to map a panel of anti-erythropoietin antibodies. Similar studies were conducted using immunoadhesins based on natriuretic peptide receptors\(^10\) and on lysosomal membrane glycoprotein 1 (LAMP-1; Ref. 79).

Ligand identification and isolation

Molecular biology approaches, such as expression cloning of cell-surface antigens and homology-based PCR cloning, have brought about the discovery of many novel molecules that resemble cell-surface receptors. Indeed, one of the most important uses of immunoadhesins has been to isolate and clone the target ligands for such ‘orphan’ receptors. For example, an immunoadhesin based on CD22, a cell-surface molecule that belongs to the Ig gene superfamily, was used to identify CD45, a lymphocyte surface glycoprotein, as a CD22 ligand\(^11\). Subsequent studies with CD22 immunoadhesins identified additional stromal-glycoprotein ligands, suggesting that CD22 is a lectin.
Immunoadhesins as potential therapeutics

Notwithstanding the value of immunoadhesins as research tools, perhaps the most exciting potential for these recombinant proteins is in human therapy. Immunoadhesins and mAbs share two important properties that are significant to their potential as therapeutic agents. First, because of structural homology, immunoadhesins exhibit a pharmacokinetic half-life in vivo that is comparable to that of humanized mAbs of similar isotype. Second, immunoadhesins can be used in a similar manner to some mAbs to modulate biochemical interactions that play key roles in pathologic processes. Given these similarities, immunoadhesins constitute a viable alternative to mAbs as a therapeutic strategy. In principle, immunoadhesins designed as antagonists to inhibit deleterious interactions, as well as immunoadhesins designed as agonists to enhance beneficial functions, hold promise as human therapeutics. Below, we review a few examples of both types.

Immunoadhesins as antiviral agents

The first immunoadhesins were designed to block the interaction of a virus with its target cell as a means of preventing infection. HIV-1 uses its gp120 envelope protein to attach to human T cells prior to infection; CD4 serves as the primary receptor for HIV attachment. Several CD4-based immunoadhesins designed to block this process were capable of binding to HIV-gp120 and, hence, of blocking the infection of target T cells in culture. Unfortunately, clinical results with CD4 immunoadhesin were disappointing, possibly as a result of differences in the in vitro and in vivo mechanisms of infection by HIV-1 (Ref. 10). More recently, novel CD4-based immunoadhesins have been described that re-target active cellular elements of the immune system against HIV-infected cells. An alternative approach is to inhibit HIV activation. Because HIV replication is enhanced by TNF, Howard et al. used a TNF-receptor immunoadhesin to block TNF-induced HIV-1 expression in infected cell lines. A different immunoadhesin with antiviral activity is based on ICAM-1, the specific receptor for rhinovirus; ICAM-1 immunoadhesin prevents rhinovirus infection of target cells in vitro. Clinical studies will be required to assess the efficacy of such immunoadhesins as antiviral agents in humans.

Immunoadhesins as inflammation and immune-function modulators

Cytokines regulate and coordinate the functions of immune and inflammatory cells to maintain homeostasis. Dysregulated activity of key cytokines perturbs homeostasis and can trigger pathological processes such as acute or chronic inflammation and autoimmune disease. An important mechanism that tightly regulates the level of activity of key cytokines is the production of soluble receptors for cytokines. Frequent, proteolytic cleavage of cell-surface receptors releases the receptor ECD, which then acts as a soluble inhibitor of its cognate cytokine. This natural inhibitory mechanism can be used therapeutically to regulate excessive cytokine activity in pathological conditions. For example, recombinant soluble TNF receptor has been administered to baboons to curtail the activity of TNF in a model of septic shock, a disease characterized by excessive inflammation. However, soluble cytokine receptors are rapidly filtered from the blood by the kidneys. To extend the pharmacokinetic in vivo half-life of soluble cytokine receptors, several groups have generated corresponding receptor-immunoadhesins. For example, immunoadhesins based on the two TNF receptors p55 (Ref. 28–30,32,33) and p75 (Ref. 34,35), the type I IL-1 receptor36, and the IFN-γ receptor α-chain37,38 have been reported. These immunoadhesins have shown protective efficacy in preclinical models of septic shock, autoimmune arthritis, inflammatory bowel disease, experimental allergic encephalomyelitis, transplant rejection and type 1 diabetes. Clinical trials of several cytokine-receptor immunoadhesins are currently under way.

A different strategy to intervene with excessive inflammation is to block the adhesion of leukocytes to the vascular endothelium and, hence, to prevent the transmigration of leukocytes into tissues, where they exert local damage. For example, inflammatory lung injury, such as in adult respiratory distress syndrome, involves a massive influx of neutrophils from the blood into the lung. The L-, E- and P-selectins play a key role in leukocyte extravasation. Immunoadhesins based on these selectins have been shown to afford protection in animal models of lung injury, and may prove to have therapeutic value.

A unique type of immunoadhesin is based on the receptor for the allergen-binding antibody IgE. Binding of IgE to high-affinity receptors on basophils and mast cells triggers mediator release, which contributes
to the allergic reaction. IgE-receptor immunoadhesin acts as a potent inhibitor of IgE binding to mast cells in vivo, and may prove to be useful for the treatment of IgE-mediated allergic disease.

Agnostic immunoadhesins based on cytokines, rather than on cytokine receptors, have also been described. IL-2 (Ref. 83) and IL-10 (Ref. 84) immunoadhesins retain the functions of their parent interleukins, but exhibit a markedly longer half-life in vivo. This type of agnostic fusion-protein broadens the spectrum of potential therapeutic applications for immunoadhesins as immunoregulatory agents.

An immunoadhesin that inhibits cancer metastasis

Proteolytic degradation of basement-membrane proteins by cell-surface urokinase is required for metastasis by a variety of human tumor cell types. Consequently, an inactive urokinase mutant expressed in tumor cell-lines completely inhibits metastasis. Alternatively, an immunoadhesin based on an enzymatically inactive mutant of urokinase can be used to inhibit urokinase activity86. This urokinase-mutant immunoadhesin reduces the metastatic capacity of a prostate cancer cell-line in mice, suggesting that inhibition of urokinase activity by this approach may be a useful strategy for combating tumor metastasis.

Practical implications

Immunoadhesins are a new class of molecules with unique applications and significant potential in human therapy. These fusion proteins are particularly useful in studies designed to investigate the biological functions of novel receptors or ligands, to define the structural requirements for ligand-receptor interactions, and to identify and isolate unknown ligands of known receptors. Moreover, preclinical studies establish an exciting potential for immunoadhesins as human therapeutic agents that may help to combat viral infection, inflammatory and auto-immune disease, and tumor metastasis. Immunoadhesins share many biological properties of mAbs, including pharmacokinetic properties and target-recognition capacity. Therefore, they may be considered as an alternative to mAbs, both in research and in therapeutics. Immunoadhesins may be preferred in certain contexts, for example, when high-affinity mAbs capable of neutralizing a given antigen are difficult to obtain but the receptor that binds to that antigen is readily available.

As more immunoadhesins and mAbs are developed and tested in clinical trials, it will be possible to assess further their comparative utility. Key areas that will need to be addressed include the feasibility of large-scale production, stability, safety, immunogenicity and pharmacokinetics and, finally, efficacy in the treatment of human disease.

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Novel antimicrobial compounds identified using synthetic combinatorial library technology

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The recent emergence of combinatorial chemistry has greatly advanced the development of biologically active lead compounds. It is anticipated that combinatorial library technology will add great value to the fight against drug-resistant bacterial strains, which pose increasingly serious health hazards. Owing to the need to use complex cell-based assays and, in turn, to screen free compounds in solution, the potential use of combinatorial libraries in the field of infectious diseases has not yet been fully explored. Despite these limitations, a number of new antimicrobial and/or antifungal compounds have been successfully identified from pools of millions of other compounds.

Following the discovery of the penicillins in the 1940s (Ref. 1), an enormous number of compounds has been screened to identify and isolate antimicrobial agents. These efforts have allowed a range of life-threatening infectious diseases, such as tuberculosis and pneumonia, to be cured. However, the widespread use of such pharmaceutical agents has led to the development of bacterial resistance to existing drugs (reviewed in Ref. 2). It is expected that this, combined with the decreasing rate of development of new antibiotics, will lead to the appearance of infectious diseases for which there is no available treatment.

Sources for novel antimicrobial compounds

Large-scale screening of individual synthetic compounds and natural products represents one of the primary approaches for the development of new antimicrobial agents. This approach primarily involves the screening of large banks of known compounds, as well as of natural sources such as soil samples, marine

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