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(72) WEISBART, Richard, US
(71) THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, US

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(54) **SYSTEME DE LIBERATION UTILISANT MAB 3E10 ET SES MUTANTS ET/OU SES FRAGMENTS FONCTIONNELS**

(54) **DELIVERY SYSTEM USING MAB 3E10 AND MUTANTS AND/OR FUNCTIONAL FRAGMENTS THEREOF**

(57) Dans le cadre de la présente invention, il a été découvert qu'une classe d'anticorps anti-ADN bicaténaire, par exemple, les mAb 3E10, lient chez l'homme les membranes des cellules rénales tubulaires fixes, pénètrent in vivo dans les cellules rénales tubulaires murines vivantes, et se localisent dans le noyau de la cellule. Les mAb 3E10 lient l'ADN bicaténaire et la porine exprimée en veinules endothéliales élevées (par exemple, en HP8/HEVIN). Des études précédentes ont porté sur les déterminants liants, tant partagés que distincts, dans les régions lourdes variables de mAb 3E10 pour l'ADN et les HP8/HEVIN. Afin d'estimer de façon indépendante les besoins en HP8/HEVIN et en ADN lors de la pénétration cellulaire, on a étudié la capacité de pénétration des mutants dirigés de mAb 3E10 des régions lourdes variables (VH) et légères variables (Vk) dans les lignées de cellules rénales. Les résultats ont montré que la pénétration des anticorps demandait la présence de résidus nécessaires pour lier l'ADN mais pas les HP8/HEVIN, ce qui indique que la pénétration cellulaire nécessite la présence de l'ADN ou d'un déterminant liant l'anticorps à la membrane qui ressemblerait exactement à l'ADN. L'anticorps Fab pénètre dans la cellule, indiquant que ni le Fc, ni la liaison d'un anticorps multivalent ne sont nécessaires pour la pénétration des cellules. L'anticorps synthétisé dans le cytoplasme et résultant de la destruction de peptides signal des chaînes lourde et légère n'est pas transloqué vers le noyau, ce qui indique la nécessité d'avoir un mécanisme d'action à médiation par la membrane ou des modifications de l'anticorps effectuées après la traduction. La présente invention démontre l'utilité de l'utilisation de mutants moléculaires et/ou de leurs fragments fonctionnels dans l'étude des mécanismes cellulaires de pénétration des auto-anticorps et de la localisation dans le noyau.

(57) In accordance with the present invention, it has been discovered that a class of anti-dsDNA antibodies, e.g., mAb 3E10, binds membranes of fixed human renal tubular cells, penetrates live murine renal tubular cells in vivo, and localizes in the cell nucleus. mAb 3E10 binds both dsDNA and an extracellular matrix protein expressed in high endothelial venules (i.e., HP8/HEVIN). Previous studies showed both shared and distinct binding determinants in the variable heavy region of mAb 3E10 for DNA and HP8/HEVIN. To independently assess the requirement of HP8/HEVIN and DNA in cellular penetration, site directed mutants of mAb 3E10 VH and Vk were investigated for the ability to penetrate kidney cell lines. The results showed that residues required for binding DNA but not HP8/HEVIN were necessary for antibody penetration, indicating that cellular penetration required the presence of DNA or binding of antibody to a membrane determinant precisely resembling DNA. Antibody Fab penetrates cells, indicating that neither the Fc nor multivalent antibody binding are necessary for cellular penetration. Antibody synthesized in the cytoplasm as a result of deleting heavy and light chain signal peptides is not translocated to the nucleus, indicating the need for a membrane mediated pathway or for post-translational modifications of the antibody. The present invention demonstrates the usefulness of using molecular mutants and/or functional fragments thereof to study the cellular pathways of autoantibody penetration and nuclear localization.

ABSTRACT OF THE DISCLOSURE

In accordance with the present invention, it has been discovered that a class of anti-dsDNA antibodies, e.g., mAb 3E10, binds membranes of fixed human renal tubular cells, penetrates live murine renal tubular cells *in vivo*, and localizes in the cell nucleus. mAb 3E10 binds both dsDNA and an extracellular matrix protein expressed in high endothelial venules (i.e., HP8/HEVIN). Previous studies showed both shared and distinct binding determinants in the variable heavy region of mAb 3E10 for DNA and HP8/HEVIN. To independently assess the requirement of HP8/HEVIN and DNA in cellular penetration, site directed mutants of mAb 3E10 VH and Vk were investigated for the ability to penetrate kidney cell lines. The results showed that residues required for binding DNA but not HP8/HEVIN were necessary for antibody penetration, indicating that cellular penetration required the presence of DNA or binding of antibody to a membrane determinant precisely resembling DNA. Antibody Fab penetrates cells, indicating that neither the Fc nor multivalent antibody binding are necessary for cellular penetration. Antibody synthesized in the cytoplasm as a result of deleting heavy and light chain signal peptides is not translocated to the nucleus, indicating the need for a membrane mediated pathway or for post-translational modifications of the antibody. The present invention demonstrate the usefulness of using molecular mutants and/or functional fragments thereof to study the cellular pathways of autoantibody penetration and nuclear localization.

DELIVERY SYSTEM USING mAb 3E10 AND MUTANTS AND/OR FUNCTIONAL FRAGMENTS THEREOF

FIELD OF THE INVENTION

The present invention relates to methods for the delivery of biologically active materials into cells, and compositions useful therefor.

BACKGROUND OF THE INVENTION

Autoantibodies to double stranded deoxyribose nucleic acid (dsDNA) are relatively specific for systemic lupus erythematosus (SLE) and are implicated in disease pathogenesis. Certain anti-DNA autoantibodies have been shown to penetrate cells and localize to the cell nucleus. Cellular penetration by anti-DNA antibodies was initially demonstrated in peripheral blood T-lymphocytes (see, for example, Okudaira, et al., in Arthritis Rheum. 30:669 (1987) and Alarcon-Segovia, et al., in Clin. exp. Immunol. 35:364 (1979)) and, subsequently, was shown to affect their function (see, for example, Okudaira, et al., supra, Alarcon-Segovia, et al., in J. Immunol. 122:1855 (1979), Alarcon-Segovia, et al., in Clin. Immunol. Immunopath. 23:22 (1982), Alarcon-Segovia and Llorente in Clin. exp. Immunol. 52:365 (1983), and Alarcon-Segovia, in Clinics in Immunology and Allergy 1:117 (1981)).

In some studies, antibody penetration was thought to be mediated by Fc receptors (see, for example, Llerena, et al., in Immunology 43:249 (1981) and Alarcon-Segovia, et al., in Nature 271:67 (1978)). For other anti-DNA antibodies, cellular penetration and translocation to the cell nucleus was thought to require the presence of DNA (see, for example, Okudaira, et al., supra). More recently, penetration of anti-DNA antibodies has been demonstrated in mesangial cells (Vlahakos, et al., in T.

Am. Soc. Nephrol. 2(8):1345 (1992)). Anti-DNA antibodies have been shown to enter the nucleus of cultured mesangial and hepatoma cells in a time and temperature dependent manner (Yanase, et al., in Lab. Invest. 71:52 (1994).

There are multiple mechanisms by which anti-DNA antibodies are thought to penetrate cells. Indeed, different antibodies may use different pathways. Since some anti-DNA antibodies have been shown to bind membrane proteins cross reactive with DNA, these proteins may be instrumental in cellular penetration (see, for example, Brentjens and Andres in Kidney Kidney International 35:954 (1989), Raz, et al., J. Immunol. 142:3076 (1989), Madaio, et al., in J. Immunol. 138:2883 (1987), Faaber, et al., in J. Clin. Invest. 77:1824 (1986), Ben-Chetrit, et al., in Clin. exp. Immunol. 60:159 (1985), Jacob, et al., in Proc. Natl. Acad. Sci. USA 81:3843 (1984), Jacob, et al., in Proc. Natl. Acad. Sci. USA 86:4669.4669 (1989), Raz, et al., in Eur. J. Immunol. 23:383.383 (1993), and Jacob, et al., in J. Clin. Invest. 75:315 (1985)). In other cases, DNA binding proteins usually thought of as intracellular have been described in association with the membrane of some cells (see, for example, Bennett, et al., in J. Clin. Invest. 76:2182 (1985) and Refeneider, et al., in Clin. Immunol. Immunopath. 63:245 (1992)). Anti-DNA antibodies could form complexes with these proteins through their mutual binding to DNA.

For additional background information, see United States Patent No. 4,812,397 and "DNA Mimics a Self-Protein That May Be a Target for Some Anti-DNA Antibodies in Systemic Lupus Erythematosus", Journal Of Immunology, pages 1987-1994 (February 15, 1995), the contents of each of which are hereby incorporated by reference in their entirety.

In view of the availability of antibodies which are capable of penetrating cells, it would be desirable to selectively utilize such cell penetrating properties for the directed manipulation of biological materials.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have identified an anti-dsDNA autoantibody (i.e., mAb 3E10, obtained from MRL/mpj/lpr mice with lupus nephritis) which penetrates renal tubular cells in vivo and localizes to the cell nucleus. In addition to binding DNA, mAb 3E10 has been discovered to bind a newly recognized protein, HP8, that is identical to an extracellular matrix protein, HEVIN, found in high endothelial venules (see Girard and Springer in Immunity 2:113 (1994)). Mutagenesis studies of the variable heavy (VH) region of mAb 3E10 reveal that DNA and HP8 share some antibody binding sites, however, other binding sites are separate and distinct for DNA and HP8. Because different mutants of mAb 3E10 differentially recognize DNA and HP8, these mutants can be utilized to determine which binding specificities are associated with cellular penetration and nuclear localization of mAb 3E10. Additional molecular constructs have been produced to evaluate the requirement for antibody constant fragment (Fc) in cellular penetration and the role of cytoplasmic proteins in nuclear localization of mAb 3E10.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for the transport of biologically active compounds into a target cell. Invention methods comprise:

combining a biologically active compound with mAb 3E10, or mutants or functional fragments thereof, and

administering the resulting combination to said cell.

In accordance with another embodiment of the present invention, there are provided methods for the transport of biologically active compounds into a target cell, said method comprising:

combining said biologically active compound with a non-pathogenic monoclonal antibody, wherein said antibody promotes transport into said cell in an energy independent manner, and wherein said antibody is not anti-RNP, and

administering the resulting combination to said cell.

Monoclonal antibodies (mAb) useful in the practice of the present invention, e.g., non-pathogenic monoclonal antibodies which promote transport into cells in an energy independent manner, and which are not anti-RNP antibodies, are capable of penetrating renal tubular epithelial cells in vivo and primary cultured neurons. Upon penetration, mAbs according to the invention (e.g., 3E10, as well as mutants and/or functional fragments thereof) localize in the cell nucleus.

In accordance with the present invention, it has been discovered that there is a class of monoclonal antibodies (e.g., mAb 3E10 and mutants and/or functional fragments thereof) which can be utilized to transport a wide variety of biologically important compounds into target cells, such as kidney cells, brain cells, ovarian cells, bone cells, and the like. Antibodies according to the invention (e.g., mAb 3E10 or mutants and/or functional fragments thereof) can be conjugated to the biological compound of interest to form an antibody conjugate which is capable of being transported into the cell. Upon entry into the cell, the antibody conjugate localizes in and

around the cell nucleus. Antibody conjugates in accordance with the present invention may be used in the same manner as other conjugated delivery systems where an antibody or other targeting vehicle is conjugated to the biological compound of interest to provide delivery to desired cells in the in vivo or in vitro environment.

Antibodies according to the invention (e.g., mAb 3E10 and mutants and/or functional fragments thereof) can be utilized to transport a wide variety of biologically active materials, e.g., nuclear transcription factors, enzymes, enzyme inhibitors, genes, and the like, to the cell nucleus for a variety of therapeutic effects. Pharmacologically active compounds including inorganic and organic compounds, pharmaceutical agents, drugs, peptides, proteins, genetic material, and the like, may be conjugated to antibodies according to the invention (e.g., mAb 3E10 and mutants and/or functional fragments thereof) for delivery thereof.

Naturally occurring antibodies are generally tetramers containing two light chains and two heavy chains. Experimentally, antibodies can be cleaved with the proteolytic enzyme papain, which causes each of the heavy chains to break, producing three separate subunits. The two units that consist of a light chain and a fragment of the heavy chain approximately equal in mass to the light chain are called the Fab fragments (i.e., the "antigen binding" fragments). The third unit, consisting of two equal segments of the heavy chain, is called the Fc fragment. The Fc fragment is typically not involved in antigen-antibody binding, but is important in later processes involved in ridding the body of the antigen.

As used herein, reference to mutants of mAb 3E10 includes variants of 3E10 which retain the same cell penetration characteristics as mAb 3E10, as well as

variants modified by mutation to improve the utility thereof (e.g., improved ability to target specific cell types, improved ability to penetrate the cell membrane, improved ability to localize to the cellular DNA, and the like). Such mutants include variants wherein one or more conservative substitutions are introduced into the heavy chain, the light chain and/or the constant region(s) of the antibody.

As used herein, reference to functional fragments of mAb 3E10 includes portions of 3E10 which retain the same cell penetration characteristics as mAb 3E10. Such functional fragments include fragments containing at least the antigen binding portion of mAb 3E10.

As readily recognized by those of skill in the art, altered antibodies (e.g., chimeric, humanized, CDR-grafted, bifunctional, antibody polypeptide dimers (i.e., an association of two polypeptide chain components of an antibody, e.g., one arm of an antibody comprising a heavy chain and a light chain, or an Fab fragment comprising V_L, V_H, C_L and C_H1 antibody domains, or an Fv fragment comprising a V_L domain and a V_H domain), single chain antibodies (e.g., a scFv (i.e., single chain Fv) fragment comprising a V_L domain linked to a V_H domain by a linker, and the like) can also be produced by methods well known in the art. Such antibodies can also be produced by hybridoma, chemical synthesis or recombinant methods described, for example, in (Sambrook et al., Molecular Cloning: A Laboratory Manual 2d Ed. (Cold Spring Harbor Laboratory, 1989); incorporated herein by reference and Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory 1988), which is incorporated herein by reference). Both anti-peptide and anti-fusion protein antibodies can be used (see, for example, Bahouth et al., Trends Pharmacol. Sci. 12:338 (1991); Ausubel et

al., Current Protocols in Molecular Biology (John Wiley and Sons, NY 1989) which are incorporated herein by reference).

A presently preferred mutant contemplated for use in the practice of the present invention is a mAb 3E10 VH mutant involving a single change of the aspartic acid residue at position 31 to asparagine (3E10-31). The preparation of this mutant and a demonstration of its cell penetration ability is set forth in Example 5. This particular mAb 3E10 mutant is especially well suited for delivery of biological compounds to kidney and brain cells. Other 3E10 mutants and/or functional fragments thereof may be used to provide targeting of biological compounds. A wide variety of mutants and/or functional fragments thereof are possible provided that they exhibit substantially the same cell penetration characteristics as mAb 3E10 and 3E10-31 after conjugation to a selected biological agent for delivery.

MAb 3E10 heavy or light chains can be produced as fusion proteins with a variety of biologically active materials, e.g., nuclear transcription factors, enzymes, enzyme inhibitors, and the like, thereby enabling the transport of these proteins into the cell nucleus of target cells. In addition, mAb 3E10 can be produced in the form of a fusion protein with other proteins that bind DNA (such as, for example, poly-L-lysine). The poly-L-lysine fusion protein with mAb 3E10 would bind DNA (e.g., plasmids containing genes of interest) and transport the DNA into the nucleus of target cells.

Fusion proteins can be designed to place the protein of interest at the amino or carboxy terminus of either the antibody heavy or light chain. Since the antigen binding fragments (Fab's) of mAb 3E10 have been shown to penetrate cells and localize in the nucleus, the entire heavy chain is not required. Therefore, potential

configurations include the use of truncated portions of the heavy and light chain with or without spacer sequences as needed to maintain the functional integrity of the attached protein.

As an alternative to producing fusion proteins as described hereinabove, a universal carrier system can be devised. For example, various proteins or DNA can be conjugated to a common carrier such as protein A, poly-L-lysine, hex-histidine, and the like. The conjugated carrier will then form a complex with antibody according to the invention. A small portion of the carrier molecule that is responsible for binding immunoglobulin could be used as the carrier. Other similar configurations include design of carriers that interact with proteins engineered into the antibody heavy or light chain.

The mode of delivery chosen for administration of antibody conjugates according to the present invention to a patient or animal will depend in large part on the particular biological compound present in the conjugate and the target cells. In general, the same dosages and administration routes used to administer the biological compound alone will also be used as the starting point for the antibody conjugate. However, it is preferred that smaller doses be used initially due to the expected increase in cellular penetration of the biological compound. The actual final dosage for a given route of administration is easily determined by routine experimentation. In general the same procedures and protocols which have been previously used for other antibody-based targeting conjugates (e.g., intravenous, intrathecal, and the like) are also suitable for the antibody conjugates of the present invention.

Many anti-DNA antibodies can penetrate several types of cells and localize to the cell nucleus. Recent

work indicates that cellular penetration requires complexes of antibody and DNA. MAb 3E10 can penetrate many different types of cell lines in tissue culture. In contrast, mAb 3E10 may be restricted in the cells it can bind and penetrate in vivo, where there is an absence of free DNA to facilitate its penetration. It appears that the penetration of mAb 3E10 into kidney cells in vivo occurs by a different mechanism by which certain antibodies can penetrate cells and localize to the cell nucleus. The use of mAb 3E10 to penetrate kidney cells and brain cells involves a mechanism which is not common to other anti-DNA antibodies which require the presence of DNA or antibody Fc binding.

Many different autoantibodies have been shown to penetrate cells, including antibodies to RNP (see, for example, Alarcon-Segovia et al. (1978) supra, Ma et al., in Clin. exp. Immunol. 93:396 (1993) and Galoppin and J. Invest. Dermatol. 76:264 (1981)), RNA (see, for example, Varesio, et al., in Cancer Res. 35:3558 (1975)), Ro (see, for example, Lee, et al., in Arthritis Rheum. 29:782 (1986)), Proteinase 3 (see, for example, Csernok, et al., in Adv. Exp. Med. Biol. 336:45 (1993)), ribosomal protein P (see, for example, Reichlin, et al., in J. Clin. Invest. 93:443 (1994) and Koren, et al., in J. Immunol. 154:4857 (1995)), lymphocytes (see, for example, Okudaira, et al., in J. Clin. Invest. 69:1026 (1982)), synaptosomes (see, for example, Fabian in Neurology 38:1775 (1988)), and neurons (see, for example, Dalmau, et al., in Neurology 41:1757 (1991) and Hormigo and Lieberman in J. Neuroizamunol. 55:205 (1994)), and some have been shown to localize in the cell nucleus. Antibodies to ribosomal protein P have been shown to penetrate pig renal cells, localize to the nucleus, and induce cell injury (see, for example, Reichlin et al., supra and Koren et al., supra), but the presence of DNA was not required.

The requirement for free DNA and the role of Fc binding for cellular penetration appears to be different for different antibodies, but the antibodies studied appear to have distinct specificities for binding antigen, and they target different cell types. Therefore, multiple mechanisms may be operative in cellular penetration and nuclear localization. In preliminary studies to determine the mechanisms for cellular penetration and nuclear localization of mAb 3E10, DNAse treatment and Fc blocking experiments were difficult to reproduce, indicating the complexity and potential artifacts involved in these procedures. Therefore, it was decided to produce mutants of mAb 3E10 variable heavy (VH) region and variable light (Vk for "variable kappa") region to definitively establish the relationship between antigen binding specificity and cellular penetration. The requirement for Fc binding and multivalent binding for cellular penetration was approached by producing molecular Fabs that are free of any contamination by undigested antibody or Fc fragments present in Fab prepared by enzyme digestion.

Monoclonal antibody 3E10 has recently been shown to cross react with a newly identified extracellular matrix protein, HP8/HEVIN (see Zack et al., in Journal of Immunology 154:1987-1994 (February 15, 1995)). However, the present studies indicate that HP8/HEVIN is not involved in the penetration of mAb 3E10 into COS-7 or 3T3 cells. Indeed, it has been unequivocally shown that cellular penetration by mAb 3E10 correlates with DNA binding but is independent of Fc binding. Moreover, multivalent binding is not required. These results suggest that cellular penetration of mAb 3E10 may occur through the formation of complexes containing antibody and DNA, but the possibility that mAb 3E10 bound to a membrane determinant that precisely resembles DNA cannot be excluded. Furthermore, the cell lines studied are penetrated by other (but not all) anti-DNA autoantibodies, suggesting a DNA dependent

mechanism of penetration that may not reflect the specificity of binding and internalization of mAb 3E10 to renal tubular cells in vivo.

In addition to cellular penetration by anti-DNA antibodies, antineuronal antibodies have been shown to penetrate neurons, and the binding of intracellular targets has been proposed as a mechanism of disease pathogenesis (see, for example, Fabian in Neurology 40:419 (1990)). Moreover, a non-immunoglobulin protein has been shown to penetrate neurons and translocate to the nucleus. A 60 amino acid polypeptide corresponding to the homeodomain of the Drosophila protein Antennapedia was recently shown to penetrate neural cells, translocate to the cell nucleus, bind DNA, and regulate neural morphogenesis (see, for example, Joliot, et al., in Proc. Natl. Acad. Sci. USA 88:1864 (1991), La Roux, et al., in Proc. Natl. Acad. Sci. USA 90:9120 (1993), Bloch-Gallego, et al., in The Journal of Cell Biology 120:485 (1993) and Derossi, et al., in J. Biol. Chem. 269:10444 (1994)). Recovery of intact peptide suggests that targeting was not to the lysosomal compartment. Sequence homology between the Antennapedia homeodomain peptide and mAb 3E10 VH or Vk is not apparent.

The mechanism for the nuclear transport of anti-DNA antibodies remains unknown, but it has been suggested that the anti-DNA antibodies might be transported to the nucleus of cells as a result of arginine-rich sequences similar to the nuclear transport signals associated with nuclear transcription factors and other proteins (see, for example, Hanover in The FASEB Journal 6:2288 (1992)). Although the amino acid sequences of mAb 3E10 VH and Vk do not show linear sequences similar to known nuclear transport signals, these signals are quite diverse and may not be easily recognized. A novel binding domain of mAb 3E10 VH that is shared only by certain anti-DNA antibodies and is composed of conserved amino acid sequences in FR1

and FR3 has been described (see, for example, Zack, et al., in Immunology and Cellular Biology 72:513 (1994)). These regions have many arginine and lysine residues that could form a nuclear transport signal by their proximity in the three dimensional structure. Therefore, mAb 3E10 has several potential determinants that could serve as nuclear transport signals. These may bind other proteins (such as the recently described hSRP1α) which act as functional receptors for some nuclear localization sequences and assist in transport across nuclear membranes (see, for example, Weis, et al., in Science 268:1049 (1995)). Alternatively, in some cases, carbohydrates are also used as nuclear transport signals (see, for example, Duverger, et al., in Exp. Cell Res. 207:197 (1993)). Glycosylation of the variable regions of the heavy or light chains could serve as a nuclear transport signal.

To assess the mechanism of nuclear transport of mAb 3E10, heavy and light chain cDNA devoid of signal peptide sequences were transfected into COS-7 cells. The engineered antibody was expressed in the cytoplasm and translocated to the cell nucleus (see, for example, Biocca, et al., in EMBO 9 (1):101 (1990)). In contrast, mAb 3E10 was not translocated from the cytoplasm to the nucleus. Therefore, either the primary sequence alone is unable to initiate transfer of the antibody into the nucleus, or transport to the nucleus utilizes a pathway initiated by binding to the cell membrane. In either case, the mechanism for the nuclear localization of mAb 3E10 may be different than the transport mechanism used for cytoplasmic proteins such as nuclear transcription factors. Since mAb 3E10 was not found in the nucleus of COS-7 cells that produced and secreted antibody, the antibody secretary and nuclear transport pathways must also be separate.

The present invention demonstrates the usefulness of specific antibodies for the introduction of biologically

active compounds into cells, as well as the usefulness of producing molecular mutants and/or functional fragments of such autoantibodies in studying the cellular pathways of autoantibody penetration and nuclear localization. Studies that elucidate the mechanisms and pathways for cellular penetration and nuclear localization of antibodies should help further the understanding of the roles of such species in health and disease, and enable the use of such materials for a variety of therapeutic applications.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1
Monoclonal Antibodies

mAbs 3E10, 5C6, and 4H2 are IgG2a anti-dsDNA antibodies which were produced from spleen cells of MRL-mpj/lpr mice by fusion with cells from the FOX-NY cell line as previously described (see Weisbart, et al., in J. Immunol. 144:2653 (1990)). mAb PP102 (Chemicon International, Temucula, CA), a murine IgG2a antibody that does not bind DNA, was used as a non-anti-DNA antibody isotype-matched control.

Example 2
Monoclonal anti-DNA antibody binding in vitro to tissues of human organs

The monoclonal antibodies were purified from ascites by affinity chromatography using protein A-Sepharose and tested for binding kidney. mAb 3E10 was also tested for the ability to bind tissues from 19 other human organs, including blood vessels, nerve trunks, liver, connective tissues, lung, pancreas, gut, cardiac muscle, striated muscle, spleen, ovary, testis, thyroid, skin, eye, adrenal, brain, pituitary, and bone. Binding of monoclonal

antibodies was detected with peroxidase conjugated affinity purified rabbit antibodies specific for mouse IgG Fc as previously described (see Taylor and Lote in Immunomicroscopy: A diagnostic tool for the surgical pathologist. Saunders, W.B., Philadelphia (1994)).

mAb 3E10 binds human renal tubular cells in vitro

Three anti-dsDNA antibodies, i.e., mabs 3E10, 5C6, and 4H2, were studied in vitro for binding tissue from fixed normal human kidney. All of the antibodies bound cell nuclei consistent with their anti-DNA reactivity. Thus, low magnification of renal tubular cells incubated with mAb 3E10 shows reactivity with renal tubular cell membranes and nuclei. Higher magnification emphasizes linear binding of mAb 3E10 to membranes of renal tubular cells. Only mAb 3E10 is seen to bind the cell surface of renal tubular cells.

The cell surface binding appeared consistent with binding the cell membrane. While mAb 3E10 bound tubular cells in five of five normal human kidneys studied, there was some variability in the intensity of staining. The results of incubating another anti-DNA antibody, mAb 5C6, with normal human kidney tubules shows binding to nuclei, but absence of binding to tubular cell membranes. The anti-DNA reactivity of mAb 5C6 is evident by the nuclear staining. However, in contrast to mAb 3E10, mAb 5C6 did not bind to renal tubular membranes.

Similarly, mAb 4H2 did not bind renal tubular membranes. None of the monoclonal anti-dsDNA antibodies were observed to bind antigens in renal glomeruli.

The specificity of binding of mAb 3E10 to kidney tubules was evaluated by studying its binding to tissues from other human organs. mAb 3E10 did not bind membranes or

cytoplasmic antigens in tissues from multiple other organs, including blood vessels, nerve trunks, liver, connective tissues, lung, pancreas, gut, cardiac muscle, striated muscle, spleen, testis, thyroid, skin, eye, adrenal, pituitary, and bone. However, results of binding ovary and brain were inconclusive.

Example 3
Monoclonal anti-DNA antibody binding in vivo to tissues of normal BALB/c mice

Normal BALB/c mice were primed with pristane and injected intraperitoneally with $2X10^7$ hybridoma cells. After two weeks, the animals developed ascites containing antibodies with anti-dsDNA reactivity. Heart, liver, and kidney tissues were obtained and preserved in liquid nitrogen for studies of tissue histology. Binding of the anti-DNA antibodies to tissues was detected with peroxidase-conjugated affinity-purified rabbit antibodies specific for mouse IgG Fc as previously described (see Taylor and Lote supra).

mAb 3E10 binds murine renal tubular cells in vivo

Thus, to determine if mAb 3E10 was reactive with mouse renal tubular cells in vivo, kidneys were examined from normal BALB/c mice two weeks after intraperitoneal injection with 3E10 cells to establish ascites containing high concentrations of mAb 3E10. mAb 3E10 did not localize in either the membrane, cytoplasm, or nuclei of liver, cardiac muscle, or renal glomerular cells. Examination of renal tubular cells, however, showed nuclear staining, indicating that mAb 3E10 was selectively internalized and transported to the nucleus in renal tubular cells in vivo. The same results were observed in three of three BALB/c mice with ascites from 3E10 cells. In contrast to sections of dead, fixed tissue incubated with mAb 3E10 in vitro

where the nuclei were exposed to the antibody, living, intact kidney cells in BALB/c mice would not be expected to contain intracellular antibody unless the antibody bound to cell membranes and entered the cells. In view of the fact that mAb 3E10 penetrated live renal tubular cells, it is likely that the cell surface staining observed in fixed dead cells could be attributed to binding the cell membrane.

The renal tubular cells from BALB/C mice with 3E10 ascites were examined in microscopic sections stained with either hematoxylin/eosin or Periodic Acid Schiff reagents. No significant abnormalities were observed.

The selective penetration of mAb 3E10 into intact renal tubular cells suggests that internalization was the result of specific antibody binding. Moreover, mAb 3E10 appears to have been transported across the nuclear membrane to localize in the nucleus in living renal tubular cells. In contrast, none of three BALB/c mice with ascites containing mAb 4H2 anti-dsDNA antibody showed binding of mAb 4H2 to liver, cardiac muscle or kidney. Comparable amounts of mAb 3E10 (0.8 mg/ml) and mAb 4H2 (1.0 mg/ml) were present in ascites used in these experiments. In contrast to mAb 4H2, mAb 3E10 enters the tubular cells and binds the cell nuclei.

These results suggest that mAb 3E10 is reactive with a membrane antigen on renal tubular cells in mouse as well as human kidney, and that the anti-kidney antibody binds renal tubular cells and is internalized and transported to the nucleus <u>in vivo.</u>

Example 4

Cell lines

MDCK dog kidney cells were kindly provided by Dr. Mostov, University of California, San Francisco, CA (see Apodaca, et al., in The Journal of Cell Biology 125:67 (1994)). Other cell lines, including 293 human embryonal kidney cells, COS-7 monkey kidney cells, NIH 3T3 cells, HT-29 colon cancer cells and LS 174T colon cancer cells were obtained from the American Type Culture Collection (ATCC, Rockville, MA). The cells were grown overnight in 96 or 48 well tissue culture plates in Dulbecco's modified Eagle media (DMEM, GIBCO BRL Life Technologies, Inc., Gaithersburg, MD) in the presence of 10% horse serum (293 cells) or fetal calf serum (remaining cell lines) at 37°C, 5% $C0_2$, and humidified atmosphere.

After one day all media were aspirated and replaced with fresh media containing monoclonal antibodies. In preliminary studies, it was determined that a 10 μg/ml concentration of purified monoclonal antibody was required to demonstrate binding to cells. In contrast, hybridoma supernatants (diluted 1:1 with fresh medium) containing 0.5 μg/ml were as effective as purified antibodies, so subsequent studies were done with purified antibodies (10 μg/ml) or hybridoma supernatants.

Cells were incubated with antibody for 1 to 2 hours and washed three times in Hanks Buffered Salt Solution and phosphate buffered saline (PBS). The cells were then fixed with 70% ethanol for 10 to 20 minutes and washed again multiple times in PBS. The cells were then incubated for 1 hour with alkaline phosphatase conjugated goat anti-mouse antibodies specific for binding IgG2a. The cells were washed three times in PBS and stained with nitroblue tetrazolium chloride/5-bromo-4-chloro-

3indolylphosphate p-toluidine salt (NBT/BCIP) in color development solution containing levamisole.

mAb 3E10 penetrates living kidney cells in tissue culture and is translocated to the nucleus

Several cell lines were examined for binding mAb 3E10. Kidney cell lines from human (293 cells), monkey (COS-7 cells), and dog (MDCK cells) were all observed to internalize mAb 3E10 and transport the antibody to the nucleus after only one hour of incubation. The kidney cells remained viable as demonstrated by the exclusion of trypan blue. In contrast, mAb 3E10 did not penetrate human colon cancer cells from LS 174T and HT-29 cell lines. Furthermore, an isotype matched control antibody, PP102, without DNA binding reactivity did not penetrate any of the kidney cell lines. Antibody penetration and nuclear localization was not observed after only 15 minutes, but it was observed as early as 30 minutes and became maximal at 60 to 90 minutes.

Example 5

Mutagenesis of mAb 3E10 Vk

The cloning of mAb 3E10 heavy and light chain cDNA was carried out as previously reported (see, for example, Zack, et al., 1994, supra, and Zack, et al., in J. Immunol. 154:1987 (1995)). Site directed mutagenesis of mAb 3E10 VH and Vk was performed by the method of Eckstein et al. (oligonucleotide--directed in vitro mutagenesis system, Amersham Corp., Arlington Heights, IL). The VH31 mutant used in these studies has been previously reported (see Zack, et al., 1995, supra). The mutated heavy and light chain cDNAs were ligated into the pSG5 expression vector (Stratagene, La Jolla, CA). Individual colonies were selected from transformed competent bacterial cells, and plasmids were prepared using the Wizard DNA

purification system (Promega Corp., Madison, WI). Mutations were confirmed by dideoxynucleotide sequencing. The oligonucleotides used for mutagenesis of mAb 3E10 Vk are listed below.

Mutation	CDR	Residue	Oligonucleotide
SVST deletion	1	27A-D	5'-TGCAGGGCCAGCAAATCTAGCTATAGT-3' (SEQ ID NO:1)
S to D	1	27C	5'-CAAAAGTGTCGATACATCTAGC-3' (SEQ ID NO:2)
Y to F	1	32	5'-AGCTATAGTTTCATGCACTGG-3' (SEQ ID NO:3)
Q to S	2	53	5'-TATGCATCCTCCCTAGAATCT-3' (SEQ ID NO:4)
R to N	3	92	5'-TCAGCACAGTAATGAGTTTCCGTG-3' (SEQ ID NO:5)
F to D	3	94	5'-CAGTAGGGAGGATCCGTGGACG-3' (SEQ ID NO:6)

Effect of a VH mutant on cell penetration by mAb 3E10

Monoclonal antibody 3E10 heavy and light chain cDNAs were transfected into COS-7 cells and secretion of mAb 3E10 by the transfected COS-7 cells was demonstrated (see, for example, Zack et al., in Journal of Immunology 154:1987-1994 (February 15, 1995)). Since mAb 3E10 can penetrate COS-7 cells, it was of interest to determine if secreted antibody was reinternalized in cultured cells. Only a small fraction of COS-7 cells produce antibody, thus the concentration of antibody in COS-7 supernatant is in the range of only 30 to 50 ng/ml. This is in marked contrast to concentrations of 10 to 50 μg/ml of purified antibody and 500 ng/ml of hybridoma supernatant required for the demonstration of antibody internalization in

previous experiments. As expected, native mAb 3E10 was detected in the cytoplasm in about 1% of COS-7 cells after transfection. However, there was no evidence of translocation to the nucleus, indicating that the pathway by which antibody is secreted bypasses the pathway for nuclear localization. Moreover, the concentration of antibody in the COS-7 cell supernatant was insufficient to detect reentry of antibody into neighboring cells.

A mutant of mAb 3E10 VH has previously been produced involving a change in residue 31 in CDR1 from aspartic acid to asparagine. This mutation increases the ability of the antibody to bind to DNA (see Zack, et al., 1995, supra). Transfection of COS-7 cells with cDNA of the native 3E10 light chain and cDNA of the VH31 mutant heavy chain resulted in an antibody that was readily observed to penetrate neighboring COS-7 cells and localize in the nucleus. The transfection efficiency was the same for the mutant and native heavy chain cDNAs, and the concentration of the mutant mAb 3E10 in COS-7 cell supernatants was the same as the native antibody.

Effect of mAb 3E10 Vk mutations on binding specificity and cellular penetration

In previous studies, mAb 3E10 was used to identify a newly recognized extracellular matrix protein, HP8, in a cDNA expression library (see Zack, et al., 1995, supra). Moreover, DNA and HP8 were shown to share multiple binding determinants on mAb 3E10 VH. In the present studies, mutations in the CDR of mAb 3E10 Vk light chain were observed to eliminate binding to both dsDNA and HP8, dsDNA alone, and HP8 alone (Table I).

Table I

Effect of mAb 3E10 VH and Vk Mutations on Binding Specificity and Cellular Penetration

mAb 3E10		Mutation		Antibody Specificity		Cell entry Cell	
H chain	L chain	Location	Change	dsDNA	HP8	COS	3T3
Native	Native			0.26	2.02	No	No
31	Native	H CDR1	D to N	3.19	1.30	Yes	Yes
31	94	L CDR3	F to D	0.50	0.20	No	No
31	27A-D	L CDR1	delete	0.13	0.10	No	No
31	92	L CDR3	R to N	0.15	1.21	No	No
31	32	L CDR1	Y to F	0.16	2.76	No	No
31	27C	L CDR1	S to D	3.22	0.26	Yes	Yes
31	53	L CDR2	Y to S	2.78	0.10	Yes	Yes
No H	No L			0.14	0.10	No	No

These results are consistent with the previous observations that dsDNA and HP8 share some but not all of binding determinants of mAb 3E10 VH. 3T3 cells have also been shown to express HP8 by Northern hybridization.

In order to further evaluate these differences, the relationship between antibody binding to dsDNA and HP8 and antibody penetration into COS-7 cells and 3T3 cells was studied. COS-7 cells and 3T3 cells were co-transfected with cDNA corresponding to the 3E10 heavy chain 31 mutant and cDNA corresponding to different kappa chain mutants. Cell penetration could not be demonstrated in antibodies containing each of four mutations in mAb 3E10 Vk that eliminated or reduced binding to dsDNA (see Table I). These mutations include residues 27A-D and 32 in CDR1, and residues 92 and 94 in CDR3. Two of the mutations, deletion of 27A-D in CDR1 and alteration of residue 94 in CDR3 eliminated antibody binding to both dsDNA and HP8. The

other two mutations, residue 32 in CDR1 and residue 92 in CDR3 removed reactivity with dsDNA but did not affect binding to HP8. If reactivity with HP8 alone was removed, as in the mutation of residue 27C or of residue 53, the mutated antibody retained the ability to penetrate cells as long as the determinants essential for dsDNA binding remained intact. These results suggest that HP8 is not involved in antibody internalization in either of these cell lines. Cellular penetration by mAb 3E10 could be due to the formation of antibody-DNA complexes, or mAb 3E10 may bind a membrane determinant that precisely resembles DNA.

Example 6
Molecular constructs of mAb 3E10 heavy and light chain cDNA

mAb 3E10 heavy and light chain cDNA without leader sequences were amplified by PCR using sense primers beginning at FR1 with the addition of the nucleotide sequence ATG. The primers used were:

Heavy chain sense primer:

5'-GCCATGGAGGTGCAGCTGGTGGAGTC-3' (SEQ ID NO:7)

Heavy chain antisense primer:

5'-AATTCTTATTTACCC(A)G(A)GAG
T(A)C(G)C(T)GGGGAA(T)(G)GC(G)TCT-3' (SEQ ID NO:8)

Light chain sense primer:

5'-GCCATGGACATTGTGCTGACACAGTC-3' (SEQ ID NO:9)

Light chain antisense primer:

5'-GAATTCTTAACACTCATTCTTGTTGAAGCTCTT-3' (SEQ ID NO:10)

To produce Fab of mAb 3E10, a heavy chain construct was amplified by PCR to contain the heavy chain leader sequence

through CH1 and terminating in a stop codon. The primers used were:

Heavy chain sense primer:

5'-ATGGACTCCAGGCTCAATTTAGTTTTC-3' (SEQ ID NO:11)

Heavy chain antisense primer:

5'-TTATTAAATTTTCTTGTCCACTTTGGTG-3' (SEQ ID NO:12)

The conditions used for PCR were: 1 minute denaturation at 95°C, 1 minute annealing at 55°C, and 1.5 minutes extension at 72°C for 38 cycles with an additional 2 second extension time per cycle.

Localization of mAb 3E10 devoid of signal peptides

To determine if mAb 3E10 is transported to the nucleus as a result of binding cytoplasmic proteins, mAb 3E10 was expressed in COS-7 cells without signal peptide sequences to prevent localization to the endoplasmic reticulum and subsequent secretion from the cell. Production of mAb 3E10 and its localization to the cytoplasm was demonstrated by histological staining using antibodies to mouse kappa chains. The failure to secrete antibody was shown by the absence of antibody in COS-7 supernatants as measured by ELISA. mAb 3E10 was localized in the cytoplasm, but it was not translocated to the nucleus. Sham transfected COS-7 cells were similarly stained using antibodies to mouse kappa chains as a control.

Penetration of cells by mAb 3E10 Fab

To investigate the requirement for antibody Fc and multivalent antibody binding in cellular penetration, the cellular penetration of mAb 3E10 Fab was examined. mAb 3E10 heavy chain cDNA, including the leader sequence, VH,

and CH1, was amplified by PCR from cDNA of mAb VH31 mutant. The amplified fragment was ligated in pSG5 and co-transfected into COS-7 cells along with mAb 3E10 light chain cDNA. Secretion of antibody Fab by COS-7 cells was confirmed by a capture ELISA with plates coated with goat antibodies to mouse gamma chains (CH1) and detected by goat antibodies to mouse kappa chains. Fab were reinternalized in neighboring cells and found localized in the nucleus as detected by antibodies to mouse gamma chains. Sham transfected COS-7 cells were similarly stained with antibodies to mouse gamma chains as a control. These results eliminate the requirement of Fc and multivalency of antigen binding for cellular penetration by mAb 3E10.

Example 7

Expression of antibodies

Purified pSG5 plasmids containing heavy and light chain gene inserts were expressed in COS-7 mammalian cells. Two micrograms each of plasmid DNA containing a heavy chain cDNA and a light chain cDNA were transfected using DEAE-dextran into 10^5 COS-7 cells grown in DMEM and 10% fetal calf serum. After three days of culture, the supernatants were harvested and tested by ELISA for the presence of light and heavy chains. The cells were fixed with 70% ethanol for 1 to 2 minutes and washed again multiple times in PBS. The cells were then incubated with alkaline phosphatase conjugated goat anti-mouse antibodies specific for binding IgG2a. The cells were washed in PBS for 3 hours and stained with NBT/BCIP in color development solution containing levamisole.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: The Regents of the University of California

(ii) TITLE OF THE INVENTION: DELIVERY SYSTEM USING mAb 3E10 AND MUTANTS AND/OR FUNCTIONAL FRAGMENTS THEREOF

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Gowling, Strathy and Henderson
(B) STREET: Suite 2600, 160 Elgin St.
(C) CITY: Ottawa
(D) PROVINCE: Ontario
(E) COUNTRY: CANADA
(F) POSTAL CODE: K1P 1C3

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows DEMONSTRATION Version 2.0D

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: to be assigned (PCT/US97/03785)
(B) FILING DATE: 07-MAR-1997
(C) CLASSIFICATION:

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(B) FILING DATE: 08-MAR-1996

(viii) AGENT INFORMATION:
(A) NAME: Eli J. McKhool
(C) REFERENCE NUMBER: 08-880910CA

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (613) 233-1781
(B) TELEFAX: (613) 563-9869

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGCAGGGCCA GCAAATCTAG CTATAGT 27

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAAAAGTGTC GATACATCTA GC 22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGCTATAGTT TCATGCACTG G 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TATGCATCCT CCCTAGAATC T 21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCAGCACAGT AATGAGTTTC CGTG 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAGTAGGGAG GATCCGTGGA CG 22

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCCATGGAGG TGCAGCTGGT GGAGTC 26

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AATTCTTATT TACCCAGAGA GTACGCTGGG GAATGGCGTC T 41

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCCATGGACA TTGTGCTGAC ACAGTC 26

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GAATTCTTAA CACTCATTCT TGTTGAAGCT CTT 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGGACTCCA GGCTCAATTT AGTTTTC 27

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTATTAAATT TTCTTGTCCA CTTTGGTG 28

That which is claimed is:

1. A method for the transport of a biologically active compound into a target cell, said method comprising:

combining said biologically active compound with mAb 3E10, or mutant or functional fragment thereof, and

administering the resulting combination to said cell.

2. A method according to claim 1 wherein said biologically active compound is a nuclear transcription factor, an enzyme, an enzyme inhibitor, genetic material, an inorganic and organic compound, a pharmaceutical agent, a drug, a peptide, or a protein.

3. A method according to claim 1 wherein said target cell is a kidney cell, a brain cell, an ovarian cell or a bone cell.

4. A method according to claim 1 wherein mAb 3E10 or mutant thereof is mAb 3E10 or mAb 3E10-31.

5. A method according to claim 1 wherein said biologically active compound and mAb 3E10, or mutant or functional fragment thereof, are covalently linked through a peptide bond.

6. A method according to claim 1 wherein said biologically active compound and mAb 3E10, or mutant or functional fragment thereof, are coadministered with a universal carrier therefor.

7. A method according to claim 6 wherein said universal carrier is protein A, poly-L-lysine or hex-histidine.

8. A method for the transport of a biologically active compound into a target cell, said method comprising administering a combination of biologically active compound and mAb 3E10, or mutant or functional fragment thereof, to said cell.

9. A method according to claim 8 wherein said biologically active compound is a nuclear transcription factor, an enzyme, an enzyme inhibitor, genetic material, an inorganic and organic compound, a pharmaceutical agent, a drug, a peptide, or a protein.

10. A method according to claim 8 wherein said target cell is a kidney cell, a brain cell, an ovarian cell or a bone cell.

11. A method according to claim 8 wherein mAb 3E10 or mutant thereof is mAb 3E10 or mAb 3E10-31.

12. A method according to claim 8 wherein said biologically active compound and mAb 3E10, or mutant or functional fragment thereof, are covalently linked through a peptide bond.

13. A method according to claim 8 wherein said biologically active compound and mAb 3E10, or mutant or functional fragment thereof, are coadministered with a universal carrier therefor.

14. A method according to claim 13 wherein said universal carrier is protein A, poly-L-lysine or hex-histidine.

15. A conjugate comprising mAb 3E10, functional fragments or mutants thereof, in combination with a biologically active compound.

16. A conjugate according to claim 15 wherein said biologically active compound is a nuclear transcription factor, an enzyme, an enzyme inhibitor, genetic material, an inorganic and organic compound, a pharmaceutical agent, a drug, a peptide, or a protein.

17. A conjugate according to claim 15 wherein mAb 3E10 or mutant thereof is mAb 3E10 or mAb 3E10-31.

18. A conjugate according to claim 15 wherein said biologically active compound and mAb 3E10, or mutant or functional fragment thereof, are covalently linked through a peptide bond.

19. A complex comprising mAb 3E10, functional fragments or mutants thereof, a biologically active compound and a carrier therefor.

20. A complex according to claim 19 wherein said carrier is protein A, poly-L-lysine or hex-histidine.

21. A method for the transport of a biologically active compound into a target cell, said method comprising:

combining said biologically active compound

with a non-pathogenic monoclonal antibody, wherein said antibody promotes transport into said cell in an energy independent manner, and wherein said antibody is not anti-RNP, and

administering the resulting combination to said cell.