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Review Article

Monoclonal Antibodies Present New Opportunities in Disease Diagnosis and Treatment

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ABSTRACT

The production of MAbs by hybridoma technology was discovered in 1975 by Georges Kohler of West Germany and Cesar Milstein of Argentina. Who developed a technique to fuse splenocyte cells (separated from the spleen of an immunized mouse) with tumorous myeloma cells., our vision for antibodies as tools for research for prevention, detection and treatment of diseases, vaccine production, antigenic characterization of pathogens and in the study of genetic regulation of immune responses and disease susceptibility has been revolutionized. Recently, MAbs have been widely applied in the field of clinical medicine. They are also used in the tissue typing, enzyme linked immunosorbent assay, radio immunoassay, immunological intervention with passive antibody, or magic bullet therapy with cytotoxic agents coupled with anti-mouse specific antibody.

Key-words: Monoclonal antibodies production- immunoassay – diagnosis - cancer- HIV-Therapeutics.

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INTRODUCTION:

Monoclonal antibodies are monospecific antibodies that are made by identical immune cells that are all clones of a unique parent cell.

Types of monoclonal antibody:

- ✓ Chimericantibody: A chime-ric antibody is a special type of therapeutic antibody made by combining genetic ingredients from a non-human animal (such as a mouse) and from a human.
- ✓ Humanized antibody: It made by only human genetic ingredient.

The development of hybridoma technology offered a number of advantages over the original art of polyclonal antibodies viz.

(i) The generation of MAbs is now a standard and increasingly routine procedure,
(ii) The use of impure antigen is tolerated since the detection of MAb of interest is largely dictated by the selection strategy,
(iii) Antibodies with selected properties or reactivities for specific structures could be selected and
(iv) The hybridoma cells line is immortal; there is an unlimited source of the MAb.

Outline procedure for monoclonal antibodies production

The hybridoma technology outline involves the isolation of spleen cells from immunized mice, their fusion with immortal myeloma cells and the production and further propagation of monoclonal antibodies from the hybrid cells

The basic strategy includes

1) Purification and characterization of the desired antigen in adequate quantity,
2) Immunization of mice with the purified antigen,
3) Culture of myeloma cells which are unable to synthesize hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) enzyme necessary for the salvage pathway of nucleic acids,
4) Removal of spleen cells from mice and its fusion with the myeloma cells,
5) Following fusion, the A hybridoma was grown in hypoxanthine aminopterin thymidine (HAT) medium. The fused cells are not affected in the absence of HGPRT unless their de novo synthesis pathway is also disrupted. In the presence of aminopterin, the cells are unable to use the de novo pathway and thus these cells become auxotrophic for nucleic acids as a supplement to HAT medium. In this medium, only fused cells will grow. Unfused myeloma cell does not have ability to grow in this HAT medium because they lack HGPRT, and thus cannot produce DNA.
7) The antibodies secreted by the different clones are then tested for their ability to bind to the antigen using an enzyme-linked immunosorbent assay (ELISA).
8) The clone is then selected for future use.

Immunoassay for testing of MAbs

Monoclonal antibodies are tested for their ability to bind to the antigen using various immunoassay .Mostly used immunoassays are:

1. Enzyme linked immunosorbent assay(ELISA)
2. Latex agglutination
3. Immune fluorescent antibody (IFA)

ANTIGEN CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY

The primary advantage of the antigen capture ELISA is the ability of this test to detect parasite antigen directly from an animal prior to or during clinical disease. The main component of the antigen capture ELISA is a MAb. The desired characteristics of this MAb are:

- Strong binding to the parasite
- The ability to attach to an ELISA plate without loss of reactivity

- In addition, a second MAb recognizing an epitope other than the MAb which is bound to the ELISA plate is often used as part of the indicator system.

Anaplasmosis, a vector-borne haemoparasiticrickettsial disease, is caused by Anaplasmamarginale and A. Centrale.Clinical disease is characterized by anemia, weight loss, abortion and death, and survivors are lifelong carriers of the rickettsia. Recently, an antigen capture ELISA has been developed for the detection of A. marginale rickettsaemia prior to the onset of clinical disease.

In addition to the detection of parasites during clinical stages of infection, it is also necessary to identify animals which have survived clinical disease and which are persistent carriers. Parasite levels in carrier animals are often too low to be detected by antigen capture ELISA; however, specific antibody is often maintained at detectable levels.

Some of the positive and negative aspects of latex agglutination, ELISA, and immune fluorescent-antibody (IFA) tests that use MAbs, as well as their respective sensitivities and specificities are as follows.

Latex agglutination is easy to perform, attractive for single-test "stat" situations (e.g., cerebrospinal fluid), provides rapid results (generally <30 min), involves very stable reagents, and has a sensitivity and a specificity lower than those of ELISA and IFA tests. On the other hand, it has a weak immunogen response for some organisms, some problems with cross-reactivity (false-positive results), is suited mostly for screening rather than quantitation, is based on subjective reading of results, and has a history of nonspecific (nonimmunogenic) agglutination.

ELISAs are rapid, highly sensitive, and specific, use a no isotopic label and stable reagents, and can be quantitated. Results are obtainable by visual observation or instrumented reading, and precise controls and small reaction volumes are used. The instrumented mode features diverse throughput capabilities and levels of sophistication. The sensitivity is greater than those of latex agglutination and IFA tests, and specificity is assay dependent. However, total automation is unavailable, preparation steps are still required (i.e., washing and aspiration),ELISAs are labor intensive, there are color discrimination problems in the visual reading mode, some enzymes are subject to interference (i.e., inhibition), and technician training is necessary.

IFA tests use stable reagents and a minimum number of steps and give rapid results. Some automation is available, e.g., FIAX, and more sophisticated application is possible (for example, time-resolved fluoro immunoassays. Sensitivity is greater than those of latex agglutination and ELISA (assay dependent), and specificity is assay dependent. On the other hand there is a lack of total automation, the tests are labor intensive, there is nonspecific (background) fluorescence interference, the sensitivity is comparable to that of ELISAs in some cases, and a trained technician is necessary.

MONOCLONAL ANTIBODIES AS RESEARCH TOOLS

Diagnosis of disease - monoclonal antibodies can be made which bind to specific antigens on blood clots or on cancer cells. The monoclonal antibodies can also carry markers which make it easy for doctors to see where they have built up. This allows doctors to detect problems before they seriously affect a person's health. For example, the blood test for prostate cancer uses monoclonal antibodies to bind to prostate-specific antigens.

Treatment of disease - monoclonal antibodies can be used to carry drugs to specific tissues. Because they bind to the antigens in a tumor, for example, they can be used to take drugs or radioactive substances directly to the cancer cells.

REDUCE MOTHER TO CHILD HIV TRANSMISSION

With the dramatic progress in effective interventions to prevent mother-to-child HIV transmission (PMTCT), new pediatric HIV infections have become rare in high-income settings. In the last five years, PMTCT interventions have been implemented and have undergone rapid scale-up in low-resource settings; leading UNAIDS to set a new goal to virtually eliminate new pediatric HIV infections by 2015. Virtual elimination has been defined as a 90% reduction in mother-to-child transmission (MTCT) from 2009 levels, to <40,000 new infections annually and an

overall transmission rate of <5% in breastfeeding populations. However, significant implementation challenges remain in the 21 priority countries, making it unlikely that the goal will be met with the existing interventions alone.

The trans placental transfer of maternal antibodies to infants protects children from infectious pathogens until immunological maturity is sufficient to produce and regulate effective immune responses. Immunoglobulin transfer continues after birth through breastfeeding, which also provides essential nutrients that are not otherwise available. Unfortunately, during chronic HIV infection the antibodies present in the infected host can generally neutralize virus from three to six months earlier, but are not able to neutralize contemporaneous circulating strains. Thus, the antibodies present in the serum of HIV-infected mothers are not sufficient to prevent infection from viruses to which infants are exposed during the intrapartum period and through breast milk. In breastfeeding infants born to HIV-1-infected mothers, overall MTCT can be as high as 40% with prolonged breastfeeding in the absence of antiretroviral (ARV) prophylaxis.

Optimal prevention requires identification of maternal HIV infection early in pregnancy with prompt initiation of ARV therapy. Studies have demonstrated that initiation of therapy later than 13 weeks before delivery is associated with increased risk of MTCT. However, women in low-resource countries may miss opportunities to reduce transmission due to missed HIV screening in antenatal settings, delivery outside of formal medical settings, HIV infection during pregnancy and breastfeeding, and the need to extend breastfeeding to provide the infant with the best overall chance of survival.

While formula feeding is recommended for HIV-infected mothers in industrialized countries, breastfeeding is the cornerstone of infant survival in many low-resource countries. In such settings, the World Health Organization (WHO) recommends that HIV-infected mothers should breastfeed for 12 months with concurrent infant or maternal ARV prophylaxis to reduce transmission risk [. However, new reports suggest that weaning prior to age 18 months is associated with elevated morbidity and mortality among HIV-exposed, uninfected children even in clinical trial settings .Additionally, the use of ARV prophylaxis by mother or infant during breastfeeding can reduce but does not eliminate transmission risk and relies on strict adherence to daily drug administration. Breakthrough infections at rates as high as 2–5% by age six months and 6% by age 12 months have been observed in breastfeeding infants of HIV-infected mothers who have been provided with triple ARV drug therapy during pregnancy and breastfeeding. Although ARV prophylaxis significantly reduces MTCT, effective implementation is complicated by the need for prolonged drug administration and adherence, potential toxicities leading to continued monitoring requirements, potential for drug resistance, and inadequate health care infrastructure. Adherence to therapy during the postpartum period has been particularly problematic for women. Since 2009, there has been a 38% decrease in new pediatric HIV-1 infections across the 21 countries in sub-Saharan Africa that account for 90% of all new pediatric infections. However, there were still 210,000 new pediatric infections in these countries in 2012, with an estimated overall transmission rate of 17% (15–20%). Approximately 40–50% of these infections were acquired through breastfeeding.

Thus, it appears unlikely that the goal of global elimination will be met with current ARV interventions alone, and continued investigation of preventive interventions to reduce MTCT, including maternal and/or infant passive/active immunization, remains important.

Monoclonal Antibodies Present New Opportunities

Starting in 2008, a technological revolution has taken place in HIV antibody research. Development of next-generation sequencing, advances in in vitro B cell clonal amplification, and high-throughput neutralization assays allow investigators to study the diversity of antibody responses in HIV-infected subjects and to identify antibodies with long-sought properties. Using RT-PCR, both the heavy and light chain immunoglobulin (Ig) genes can be amplified from single B cells and cloned into expression vectors. These techniques allow large-scale isolation and synthetic production of human monoclonal antibodies (MAb) by transfection of producer cells *in vitro*. As a result, the field that had identified only four broadly neutralizing antibodies (bNAbs) in the past 25 years has suddenly

found dozens of new bNAbs, some of which were two orders of magnitude more potent, with much greater breadth than their predecessors. Most of the HIV-1-specific bNAbs found to date have unusual properties including long heavy chain CDR3 loops, extensive somatic hypermutation, and glycan dependence, which may explain why it has been hard to induce bNAbs by vaccination and why the neutralizing antibody response to natural infection lags behind the evolution of neutralization-resistant genetic variants. Whether the newly discovered antibodies could be used to treat or prevent HIV-1 infection is a question that is being actively explored. Historically, passively administered antibodies have been shown to have an excellent safety profile and to protect against multiple viruses, including cytomegalovirus, varicella zoster virus, poliovirus, hepatitis A and B viruses, measles virus, Junín virus, rabies virus, and respiratory syncytial virus (RSV). Polyclonal immunoglobulins have also been shown to be effective in preventing perinatal transmission of several viruses. For example, cytomegalovirus hyper immune globulin administered to pregnant mothers infected with cytomegalovirus during pregnancy was shown to reduce perinatal transmission by 50% .Before the vaccine for hepatitis B was available, the recommended intervention to prevent perinatal hepatitis B transmission was to administer hepatitis B immunoglobulin to all infants born to hepatitis B surface antigen positive mothers at zero, three, and six months after birth ,even with active hepatitis B vaccination, a single dose of hepatitis B immunoglobulin continues to be recommended. Administration of varicella zoster hyper immune globulin to infants born to mothers with varicella around the time of delivery was also shown to reduce the chances of perinatal transmission of varicella zoster virus. Compared to polyclonal immunoglobulin preparations, mAbs are considered to be safer because they are not derived from blood products. Several mAbs are now in clinical development, and two mAbs are currently licensed for use against the microbial pathogens RSV and anthrax.

Clinical Path to Testing Efficacy in Infants

The primary objective for passive administration of a monoclonal bNAb to infants would be to prevent infection. The mAb would therefore be given to the infant immediately after delivery with the hope of preventing some intrapartum transmission events and to establish adequate levels of neutralizing activity in the infant's serum to prevent breast milk transmission. The mAb would specifically not be administered to mothers to avoid the possibility of producing neutralization-resistant variants. Before performing these studies in high-risk infants, a series of safety and pharmacokinetic studies will need to be done in adults. Figure 2 outlines one potential sequence of trials that would provide the safety and pharmacokinetic data needed to support a phase IIb efficacy trial, and would also provide logistical data to inform the feasibility analysis being done in parallel as outlined below. Evaluation in HIV-uninfected adults could proceed to testing a small cohort of HIV-exposed infants before determining the dose and schedule for a phase IIb test-of-concept efficacy trial in high-risk infants. Studying the mAb in HIV-infected adults would be informative for two major reasons. First, because some infants may be unknowingly infected at birth, safety data for mAbs in the presence of HIV infection will be needed. Second, the impact on viral replication and pathogenesis could be assessed to indirectly support the primary objective of the prevention trial. Because the typical half-life of a mAb *in vivo* is 21–24 days, the dosing would be monthly and continue until the completion of breastfeeding. Therefore, multi-dose safety data from early phase trials will be needed. These studies would take up to 18 months and require careful planning and coordination with clinical investigators and stakeholders at trial sites, in addition to ongoing communication with and guidance from regulatory agencies.

Figure 1. One possible clinical pathway for testing monoclonal antibodies in infants.

Safety, pharmacokinetics (PK), and biological effect on virus populations are initially assessed in HIV-positive adults. Dosing is further refined in studies in HIV-negative adults and infants. This information is used to design an efficacy study in HIV-exposed infants as an adjunct to standard ARV treatment and prophylaxis.

Challenges for Conducting Efficacy Trials

Passive immunization is proposed to complement and improve upon the existing PMTCT interventions. Therefore, in clinical trials the mAbs must be tested in combination with local standard of care for HIV prevention. Our estimates show that, with currently observed rates of pediatric HIV-1 infections in infants born to HIV-infected mothers who receive antiretroviral therapy starting either late in pregnancy or postpartum (estimated to be 3–5% based on several clinical trials, a study would need to enroll 1,000–3,000 mother-infant pairs to be able to detect efficacy of 60% or more for the adjunct intervention. The variability in the required sample size depends on the expected transmission rate, which will be dependent on inclusion criteria that determine the level of transmission risk, and on estimated attrition rate. One of the key populations that would need to be enrolled in the study is women presenting late to antenatal clinics: that is, women with a high risk of transmission even with optimal ARV intervention. Recruitment into the trial will have to be done during the short time between their arrival and delivery, a time when they are particularly vulnerable and may be dealing with the HIV diagnosis. Retaining these women in a trial that requires multiple, regular visits will also be challenging because late presentation is usually caused by socioeconomic disenfranchisement and difficulty accessing medical care.

Cost Considerations

MAbs are expensive to produce, and it has been argued that the product would not be affordable in resource-limited settings for the population that will need it most. However, any cost-benefit analysis should compare the short-term costs of delivering the prophylaxis with the costs of lifelong treatment and care for an HIV-infected infant. These costs vary widely from one setting to another and will continue to decrease in the future, as both the costs for treatment and for bulk production of antibodies are rapidly improving.

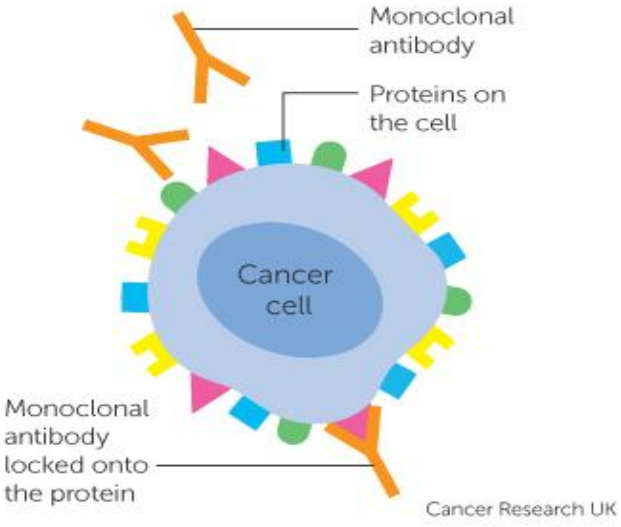
Feasibility for Integration with Current Standard of Care

The goal of product development should be to seek a target profile that will be feasible with minor changes in the countries' health care infrastructure. Passive immunization would provide protection that that does not depend on strict daily adherence to ARV drugs; thus, passive immunization would be an important adjunct to increase efficacy of current ARV regimens. However, it requires the product to be regularly administered parenterally by qualified medical personnel and, therefore, attention would need to be paid to injection safety, a reliable supply chain, and a plan for medical waste. Required frequency of administration will be a critical factor in product development. Pharmacodynamic properties of VRC01 suggest that administration will be needed every four weeks to maintain the concentration that is expected to be protective. Even if these administrations are timed to coincide with routine infant immunization schedules as much as possible, additional medical visits would be required, putting additional burden on families and health care systems. The situation is further complicated by the fact that many of the infants who would benefit most from this intervention live in communities with limited access to routine health care. The first translation efforts are focusing on proof-of-concept studies, but for optimal implementation, final products should be effective with less frequent administration. Fortunately, new approaches are being developed to extend the half-lives of monoclonal antibodies in vivo.

MONOCLONAL ANTIBODIES USED IN CANCER TREATMENT

Monoclonal antibodies recognise and attach to specific proteins produced by cells. Each monoclonal antibody recognises one particular protein. They work in different ways depending on the protein they are targeting. So different monoclonal antibodies have to be made to target different types of cancer.

Many different monoclonal antibodies are already available to treat cancer. Some are licensed to treat particular types of cancer. Some newer types are still in clinical trials. Different monoclonal antibodies cause different side effects. It can take a long time to develop this type of treatment because making monoclonal antibodies can be very complicated



Monoclonal antibodies work in different ways and some work in more than one way. Based on work its classify:

- Trigger the immune system to attack cancer cells
- Block molecules that stop the immune system working (checkpoint inhibitors)
- Block signals telling cancer cells to divide
- Carry cancer drugs or radiation to cancer cells

Trigger the immune system
Some monoclonal antibodies trigger the immune system to attack and kill cancer cells. Some monoclonal antibodies simply attach themselves to cancer cells, making them easier for the cells of the immune system to find them.

Blocking molecules that stop the immune system working (checkpoint inhibitors)
The immune system uses particular molecules to stop it being over activated and damaging healthy cells. These are known as checkpoints. Some cancer cells make high levels of checkpoint molecules to switch off immune system cells called T cells. T cells would normally attack the cancer cells. Drugs that block checkpoint molecules are called checkpoint inhibitors. They are a type of immunotherapy and include drugs that block CTLA-4, PD-1 and PD-L1 (programmed death ligand 1).

Block signals telling cancer cells to divide
Cancer cells often make large amounts of molecules called growth factor receptors. These sit on the cell surface and send signals to help the cell survive and divide. Some monoclonal antibodies stop growth factor receptors from working properly. Either by blocking the signal or the receptor itself. So the cancer cell no longer receives the signals it needs.

Carry cancer drugs or radiation to cancer cells
Some monoclonal antibodies have drugs or radiation attached to them. The MAB finds the cancer cells and delivers the drug or radiation directly to them. These are called conjugated MABs

APPLICATION OF MONOCLONAL ANTIBODY
MAbs have proved to be extremely valuable for basic immunological and molecular research because of their high specificity. They are used in human therapy, commercial protein purification, suppress-ing immune response, diagnosis of diseases, cancer therapy, diagnosis of allergy, hormone test, purification of complex mixtures, structure of cell membrane, identification of specialized cells, preparation of vaccines, and increasing the effectiveness of medical substance

Table: Examples of recently introduced therapeutic antibody

NAME	TRADE NAME	TYPE	SOURCE	TARGET	USE
Abagovomab		mab	mouse	CA-125	Ovarian cancer
Abituzumab		mab	humanized	CD51	Cancer
Abrilumab		mab	human	Integrinα4β7	Inflammatory bowel disease, ulcerative colitis
Adalimumab	Humira	mab	human	TNF-α	Rheumatoid arthritis, hemolytic disease of the newborn
Adecatumumab		mab	human	EpCAM	Prostate and breast cancer
Aducanumab		mab	human	Beta - amyloid	Alzheimer's disease
Alirocumab		mab	human	PCSK9	hypercholesterolemia
Anetumab ravtansine		mab	human	MSLN	Cancer
Arcitumomab	CEA-Scan	Fab	mouse	CEA	Gastrointestinal cancer
Atezolizumzb		mab	human	CD274	Cancer
Atlizumab	Actemra, RoActemra	mab	humanized	IL-6 receptor	Rheumatoid arthritis
Bapineuzumab		mab	humanized	Beta amyloid	Alzheimer's disease
Basiliximab	simulect	mab	chimeric	CD25(α chain of IL-2 receptor)	Prevention of organ transplant rejection
Bavituximab		mab	chimeric	Phosphatidylserine	Cancer , viral infection
Bectumomab	lymphoScan	Fab	mouse	CD22	Non-hodgkin lymphoma
Benralizumab		mab	humanized	CD125	asthma
Blosozumab		mab	humanized	SOST	osteoporosis
Cantuzumab ravtansine		mab	humanized	MUC1	cancers
Capromab pendetide	Ptostascint	mab	mouse	Prostatic carcinoma cells	Prostate cancer(detection)
CR6261		mab	human	Influenza a hemagglutinin	Infectious disease \ influenza A
Daratumumaba		mab	human	CD38(cyclic ADP ribose hydrolase)	cancers
Ecromeximab		mab	chimeric	GD3 ganglioside	Malignant melanoma
Exbivirumaba		mab	human	Hepatitis B surface antigen	Hepatitis B
Felvizumab		mab	humanized	Respiratory syncytial virus	Respiratory syncytial virus infection
Fezakinnumab		mab	humanized	IL-22	Rheumatoid arthritis, psoriasis
Fontolizumab	HuZAF	mab	humanized	IFN-gama	Crohn's disease
Fulranumab		mab	human	NGF	pain
Gomiliximaba		mab	chimeric	CD23(IgE	Allergic asthma

				receptor)	
Ibalizumab		mab	humanized	CD4	HIV infection
Lambrolizumab		mab	humanized	PDCD1	Antineiplastic agent
Otelixizumab		mab	Chimeric\humanized	CD3	Diabetes mellitus type 1
PRO 140			humanized	CCR5	HIV infection
Trastuzumab	Herceptin	mab	humanized	HER2\neu	Breast cancer

SUMMARY

The ability to produce monoclonal antibodies of defined antigenic specificity in unlimited quantities represents a milestone in immunological research. Conventional polyclonal antisera are produced by repeated immunization of animals such as rabbits, sheep, or goats. . In 1975 Kohler and Milstein' fused primed B cells from immunized mice with an immortal myeloma cell line of the same species, and after selective cloning produced monoclonal originally grown in selective media so that the cells became deficient in the purine enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT the enzyme deficiency of Lesch-Nyhan syndrome).2 These lines, if grown in media containing hypoxanthine, aminopterin, and thymidine, will not survive but if the enzyme is supplied by the fused normal B cell partner, then the fused cells have a selective advantage. Theoretically any antigen of interest can be used for immunisation and, in fact, monoclonal antibodies to cell surface glycoproteins, enzymes, drugs, bacterial cell surfaces, and virus components have all been raised. These monoclonal antibodies have made an enormous impact on research and diagnosis in all aspects of laboratory medicine and hold considerable therapeutic potential.

CONCLUSION

Hybridoma technology has had and will continue to have a major impact on the diagnosis (and treatment) of human disease.MAb-based ELISAs have tremendous potential for use in the diagnosis of parasitic diseases. The antigen capture ELISA can directly detect the presence of a parasite during the clinical stages of disease when treatment is most efficacious, and the C-ELISA format can be used to detect carriers of persistent parasitic infections. A final and important attribute of the C-ELISA is the ease with which it can be used with recombinant antigens. This is especially important in those parasitic diseases for which diagnostic antigen cannot yet be produced in cell culture. Similarly, in time, human monoclonal with specificities to tumor antigens may be produced and these would be immensely powerful tools in the in vivo diagnosis of small malignancies and also in the selective treatment of these tumors.

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