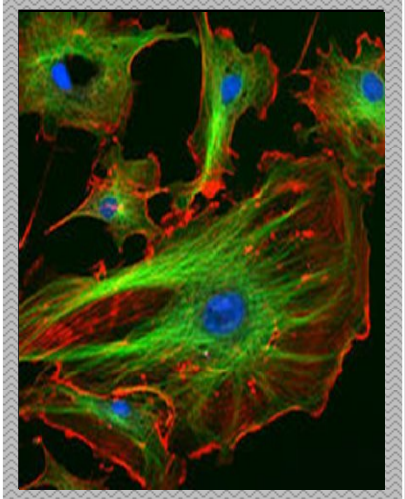
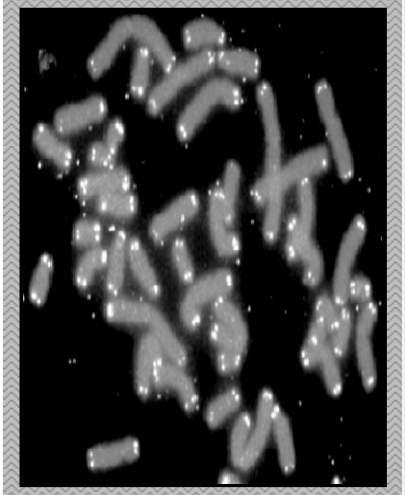
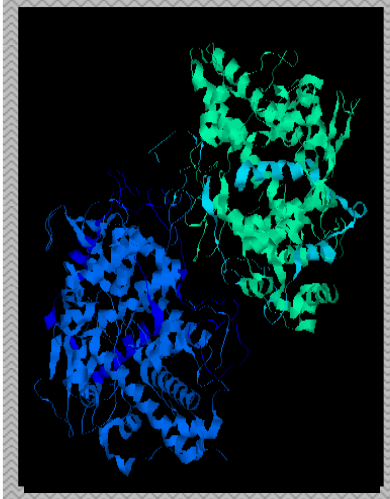






EMGEN Newsletter

Vol. 3 Issue 5, September 2009

INSIDE THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMHGBN) was created in 2004 with collaboration of representatives of selected centre of excellence in (health related) molecular biology, biotechnology & genomics in the Eastern Mediterranean region by recommendations and efforts of WHO/EMRO.

Address:
Biotechnology building, #69, Pasteur Ave., Pasteur Institute of Iran
Tehran, Iran, 13164
Tel: +98-21-66954324
Fax: +98-21-66465132
E-mail: emhgbn@gmail.com, secretariat@emhgbn.net
Website: www.emhgbn.net

Prepared by: Dr. R. Kazemali
Page Design: S. Karimzadeh, M. Tohidi
Editor: Dr. S. Sardari

Evaluation of *Leishmania infantum* rK26 antigen for Serodiagnosis of Visceral Leishmaniasis

The article entitled "Evaluation of leishmania infantum rk26 antigen for serodiagnosis of visceral Leishmaniasis" focuses on recombinant k26 antigen from Leishmania infantum and Evaluation its performance for serodiagnosis of visceral leishmaniasis. The corresponding author of this article Dr. Safar Farajnia is a researcher unit of Biotechnology Research Center , Tabriz university of Medical Sciences , Iran. This article is the result of a research which is summarized and made available to us by author for our newsletter more details of it was published in the Journal Cambridge University Press, 2008 June. 135(9): 1035 -1041

Dr. Safar Farajnia

The protozoan parasite *Leishmania* consists of three different forms of disease: cutaneous, mucocutaneous, and visceral leishmaniasis. Visceral leishmaniasis (VL), also called Kala azar, is the most severe form of leishmaniasis originated kinds of *Leishmania (L.) donovani* complex. VL influences more than 2 million people in 47 countries approximately 500,000 new cases yearly worldwide (Guerin et al., 2002). Zoonotic VL is common in many Mediterranean basin countries including Iran where causative agent is *L. infantum*. There is no efficient vaccine available against VL and development of rapid and accurate diagnostic methods is of research priorities for the disease control.

Diagnosis of VL is made classically by demonstration of *Leishmania* amastigotes in tissue samples. (Burns et al., 1993), however, the technique is invasive and limited by low sensitivity (Sundar and Rai, 2002). Anti-leishmanial antibody titers are typically high during the acute VL, and this feature has been exploited for the serodiagnosis of VL using different methods (Bogdan et al., 1990; Kumar et al., 2001). The direct agglutination test (DAT) is famous in many countries but distinction between batches of antigen and cross-reaction with some other infectious agents are of limiting factors. Different recombinant antigens have been utilized for use in serodiagnosis of VL (Carvalho et al., 2002; Maalej et al., 2003; Rosario et al., 2005). Recently a promising diagnostic antigen, known as K26 antigen, identified and characterized from *Leishmania chagasi* (Bhatia et al., 1999) and shown that rK26 ELISA has sensitivity even more than rK39, especially, for detection of VL in the early stage of infection (Sundar et al., 2002; Rosati et al., 2003). However, there is no available data about K26 antigen in other VL agents including *L. infantum*.

The goals of this study were to illustrate the *k26* gene of *L. infantum* and to prepare and assess the performance of rK26 ELISA for serodiagnosis of VL caused by *L. infantum* in endemic areas of Iran.

Three different groups of sera were screened in this study. Group A; sera from 93 parasitologically confirmed VL cases, group B; sera from 100 healthy controls and group C; sera from patients with tuberculosis (n = 10), brucellosis (n = 10) and toxoplasmosis (n = 10).

L. infantum were cultured at 26 ºC in RPMI 1640 medium with glutamine, supplemented with 10% heat-inactivated fetal calf serum. Organisms were harvested in logarithmic phase and used for DNA isolation as

The k26 open reading frame of *L. infantum* was amplified by PCR.

The PCR product was ligated into the Bluescript vector resulted into the plasmid clone pBsc-Li-k26. Nucleotide and predicted protein sequences of Li-k26 were compared to data available by the BLAST search method.

The coding region for *L. infantum k26* gene was removed from pBsc-Li-k26 clone and subcloned into the PQE 80L expression vector yielding the plasmid subclone PQ-Li-k26. The expression construct was transformed into *E. coli DH5a* strain and Protein expression was analysed by SDS-PAGE after induction with IPTG. For purification of recombinant protein, recombinant bacteria was cultured in 500 ml LB media and rK26 protein was purified on Ni-NTA column as described (Farajnia et al., 2005).

The stationary-phase promastigotes of *L. infantum* suspended in 10 mM Tris-HCl buffer (pH 7.5) at a concentration of 1×10^7/ml. The suspension was and stored in aliquots at –70°C until use.

ELISA plates (Nunc, Maxisorb) were coated overnight at 4 °C with 100 ng of rK26 or 500 ng of promastigote lysate in 0.1 M bicarbonate buffer (pH 9.0). The wells were blocked, washed three times with PBS-T, and incubated with patients sera at a 1/200 dilution. Wells were washed and incubated with Rabbit anti-human IgG conjugated with HRP for 1 h. This step was followed by three rinses with PBS-T, developed using Tetramethylbenzidine as substrate and the optical density (OD) was measured at 450.

The DAT was performed essentially as described previously (el Harith et al., 1998). Twofold serial dilution of sera were made was incubated with DAT antigen. Antibody titer of 1:3200 and above was considered as positive. PCR amplification of the coding region for *L. infantum k26* gene was resulted in a 750 bp product. The PCR product was cloned and its sequence was submitted to the GeneBank / NCBI Data Base under accession number DQ192034. Comparison of predicted amino acid sequence of *L. infantum* K26 protein with sequences in database revealed that *L. infantum k26* gene has 99 % homology to *L. Chagasi k26* sequence, whereas its homology to related gene from *L. donovani* is only 85%.

Evaluation of reactivity of VL patient's sera with *L. infantum* rK26 antigen

As shown in tables 1 and 2, the sensitivity of rK26 ELISA (90/93, 96.8 %) was higher than whole cell ELISA (89/93, 95.7%) and especially DAT (85/93, 91.4%), but differences were not statistically significant ($P > 0.05$). Analysis of performance of the three tests for serodiagnosis of VL indicated the presence of a good agreement between rK26 ELISA and whole cell ELISA ($K= 0.739$, $P = 0.000$);Whole Cell ELISA and DAT ($K= 0.753$, $P = 0.000$) and a moderate agreement between rK26 ELISA and DAT ($K= 0.526$, $P = 0.000$). However, significant differences were observed in comparing specificity of rK26 ELISA with specificity of whole cell ELISA ($P = 0.012$) and DAT ($P = 0.016$). rK26 ELISA were negative in all of 130 non-VL cases (specificity = 100%), whereas whole cell ELISA was negative in 114 cases (specificity = 87.7 %) and DAT was negative in 116 cases (specificity = 89.2).

Table1. Reactivity of sera from symptomatic VL patients, healthy controls and patients with other infectious diseases with different antigen preparations

	VL patients (N = 93)		Healthy controls (N =100)	
	+	-	+	-
Whole Cell ELISA	89	4	12	88
DAT	85	8	11	89
rK26 ELISA	90	3	0	100

VL= Visceral Leishmaniasis, DAT= Direct Agglutination Test

Table2. The percent of sensitivity, specificity, positive predictive value, and negative predictive value of DAT, Whole Cell ELISA and rK26 ELISA tests for serodiagnosis of symptomatic VL

	Sn	Sp	Ef
Whole Cell ELISA	95.7	87.7	91
DAT	91.4	89.2	90.1
rK26 ELISA	96.8	100	98.6

Sn=Sensitivity, Sp= Specificity PPV= Positive predictive value, NPV= Negative predictive value, and Ef= Efficiency

Serological methods have proven to be useful non-invasive methods for diagnosis of VL that could have epidemiological applications for determining carrier rates. But, the performance of these methods is highly dependent on the type, source, and purity of the antigens employed. In our study, DAT was positive with 91.4 % of confirmed VL cases, whereas promastigote whole cell ELISA produced positive result with 95.7 % of VL sera. However, numerous false-positive reactions were observed with both methods. DAT was positive with 11 of 100 healthy sera, 2 of ten tuberculosis sera and one of ten toxoplasmosis sera with overall specificity of 89.2 %. Whole cell ELISA was positive with 12 of 100 healthy sera, two of ten tuberculosis sera and one of ten toxoplasmosis sera with overall specificity of 87.7 %. This observation is in agreement with several reports (Choudhry et al., 1990; Singh et al., 1995; Kumar et al., 2001) and confirms that application of whole cell *Leishmania* antigens can result in false- positive results. When ELISA was performed using rK26 antigen, 96.8 % of sera from confirmed VL cases were positive. Sera from healthy controls and non-VL patients did not show any reaction with rK26 ELISA and the specificity of the test was 100 %. These results show that K26 protein is highly antigenic protein that is specific to VL agents.

This investigation received financial support from joint WHO Eastern Mediterranean Region (EMRO), Division of Communicable Disease (DCD) and the WHO Special Program for Research and Training in Tropical Disease (TDR): The EMRO/TDR Small Grants Scheme for Operational Research in Tropical and other Communicable Disease.

References

1. A. Bahatia, N.S. Daifalla, S. Jen, R. Badaro, S.G. Reed, Y.A. Skeiky, Cloning, characterization and serological evaluation of K9 and K26: two related hydrophilic antigens of Leishmania chagasi: Molecular and Biochemical Parasitology (1999). Vol. 102(2): 249-261.

2. C. Bogdan, N. Stosiek, H. Fuchs, M. Rollinghoff, W. Solbach, Detection of potentially diagnostic leishmanial antigens by Western blot analysis of sera from patients with kala-azar or multilesional cutaneous leishmaniasis. Journal of Infectious Disease (1990). Vol. 162(6): 417-1418.

3. J.M. Burns, J.r. Shreffler, W.G. Benson, D.R. Ghalib, H.W. Badaro, S.G. Reed, Molecular characterization of a kinesin-related antigen of Leishmania chagasi that detects specific antibody in African and American visceral leishmaniasis. Proceedings of the National Academy of Sciences USA (1993).Vol. 90(2): 775-779.

4. S. Farajnia, M.H. Alimohammadian, N.E. Reiner , M. Karimi , S. Ajdari, F. Mahboudi, Molecular characterization of a novel amastigote stage specific Class I nuclease from Leishmania major. International Journal for Parasitological (2004).Vol. 34(8): 899-908.

5. S. Farajnia, F. Mahboudi, N.E. Reiner, A. Kariminia, M.H. Alimohammadian, Mononuclear cells from patients recovered from cutaneous leishmaniasis respond to Leishmania major amastigote class I nuclease with a predominant Th1-like response. Clinical and Experimental Immunology (2005). Vol. 139(3): 498-505.

6. P.J. Guerin, P. Olliaro, S. Sundar, M. Boelart, S.L. Croft, P. Desjeux, M.K. Wasunna, A. Bryceson, Visceral leishmaniasis: current status of control, diagnosis and treatment, and a proposed research and development agenda. Lancet Infectious Disease (2002).Vol. 2(8): 494-501.

7. R. Kumar, K. Pai, K. Pathak, S. Sundar, Enzyme-Linked Immunosorbent Assay for Recombinant K39 Antigen in diagnosis and Prognosis of Indian Visceral Leishmaniasis. Clinical and Diagnostic Laboratory Immunology (2001).Vol. 8(6): 1220-1224.

8. I.A. Maalej, M. Chenik, H. Louzir, A. BenSalah, C. Bahloul, F. Amri, K. Dellagi, Comparative evaluation of ELISA's based on ten recombinant or purified Leishmania antigens for the serodiagnosis of mediterranean visceral leishmaniasis. American Journal of Tropical Medicine and Hygiene (2003).Vol. 68(3): 312-320.

9. E.Y. Rosário, O. Genaro, J.C. Franca-Silva, R.T. da Costa, W. Mayrink,A.B. Reis, M. Carneiro,Evaluation of enzyme-linked immunosorbent assay using crude Leishmania and recombinant antigens as a diagnostic marker for canine visceral leishmaniasis. Memórias do Instituto Oswaldo Cruz (2005). Vol. 100(2): 197-203.

10. S. Rosati, M. Ortoffi, M. Profiti, A. Mannelli, W. Mignone, E.Bollo, L. Gradoni, Prokaryotic expression and antigenic characterization of three recombinant Leishmania antigens for serological diagnosis of canine leishmaniasis. Clinical and Diagnostic Laboratory Immunology (2003). Vol. 10(6): 1153-1156.

11. S. Singh, A. Gilman-Sachs, K.P. Chang, S.G. Reed, Diagnostic and prognostic value of K39 recombinant antigen in Indian leishmaniasis. Journal of Parasitology (1995).Vol. 81(6):1000-1003.

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis, shortened as 2-DE or 2-D electrophoresis, is a form of gel electrophoresis usually used to analyze proteins. Mixtures of proteins are separated by two properties in two dimensions on 2D gels.

Basis for separation

2-D electrophoresis begins with 1-D electrophoresis but then separates the molecules by a second property in direction 90 degrees from the first. In 1-D electrophoresis, proteins (or other molecules) are divided in one dimension, so that all the proteins/molecules will lie along a lane but be separated from each other by a property (*e.g.* isoelectric point). The result is that the molecules are spread out across a 2-D gel. Because it is improbable that two molecules will be alike in two dissimilar properties, molecules are more efficiently separated in 2 -D electrophoresis than in 1-D electrophoresis. The two dimensions that proteins are separated into using this technique can be isoelectric point, protein complex mass in the native state, and protein mass. To separate the proteins by isoelectric point is named isoelectric focusing (IEF). Thereby, a gradient of pH is applied to a gel and an electric potential is applied across the gel, making one end more positive than the other. At all pHs other than their isoelectric point, proteins will be charged. If they are positively charged, they will be pulled towards the more negative end of the gel and if they are negatively charged they will be pulled to the more positive end of the gel. The proteins applied in the first dimension will move along the gel and will accumulate at their isoelectric point; that is, the point at which the overall charge on the protein is 0 (a neutral charge). For the analysis of the functioning of proteins in a cell, the knowledge of their cooperation is crucial. Most often proteins act jointly in complexes to be entirely functional. The analysis of this sub-organelle organization of the cell needs methods keeping the indigenous state of the protein complexes. In indigenous polyacrylamide gel electrophoresis (native PAGE), proteins stay in their native state and are separated in the electric field following their mass and the mass of their complexes respectively. To gain a separation by size and not by net charge, as in IEF, a further charge is shifted to the proteins by the use of coomassie or lithium dodecyl sulfate (LDS). After completion of the first dimension the complexes are wiped out by applying the denaturing SDS-PAGE in the second dimension, where the penchod proteins of which the complexes are composed of are separated by their mass. Before separating the proteins by mass, they are treated with sodium dodecyl sulfate (SDS) along with other reagents (SDS-PAGE in 1-D). This denatures the proteins (that is, it unfolds them into long, straight molecules) and joins a number of SDS molecules approximately proportional to the protein's length. Because a protein's length (when unfolded) is approximately proportional to its mass, this is comparable to saying that it attaches a number of SDS molecules approximately proportional to the protein's mass, because the SDS molecules are negatively charged, the consequence of this is that all of the proteins will have just about the same mass-to-charge ratio as each other. Additionally, proteins will not transfer when they have no charge (a result of the isoelectric focusing step) so the coating of the protein in SDS (negatively charged) permits movement of the proteins in the second dimension. In the second dimension, an electric potential is again applied, but at a 90 degree angle from the first field. The proteins will be attracted to the more positive side of the gel relatively to their mass-to-charge ratio. As formerly clarified, this ratio will be just about the

same for all proteins. The proteins' progress will be slowed by frictional powers. The gel thus acts like a molecular strainer when the current is applied, separating the proteins on the basis of their molecular weight with larger proteins being kept higher in the gel and smaller proteins being able to pass through the strainer and achieve lower regions of the gel. The result of this is a gel with proteins spread out on its surface. These proteins can then be distinguished by a variety of means, but the most frequently used stains are silver and coomassie staining. In this case, a silver colloid is applied to the gel. The silver binds to cysteine groups within the protein. The silver is dimed by exposure to ultra-violet light. The darkness of the silver can be attributed to the amount of silver and so the amount of protein at a given location on the gel. This measurement can only give estimated amounts, but is sufficient for most purposes.

Molecules other than proteins can be separated by 2D electrophoresis. In supercoiling assays, coiled DNA is separated in the first dimension and denatured by a DNA intercalator (such as ethidium bromide or the less carcinogenic chloroquine) in the second. This is similar to the integration of native PAGE /SDS-PAGE in protein separation. In summary 2D provides resolution according to two features, whereof one is most often molecular charge. The investigated molecule needs not be protein.

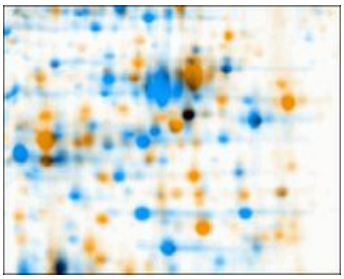


Figure 1: Warping: Images of two 2D electrophoresis gels, overlaid with Delta2D. First image is colored in orange, second one colored in blue. Due to running differences, corresponding spots do not overlap.

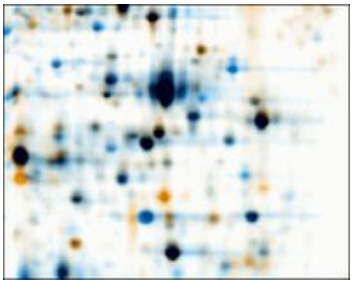


Figure 2: Warping: Images of two 2D electrophoresis gels after warping. First image is colored in orange, second one colored in blue. Corresponding spots overlap after warping. Common spots are colored black, orange spots are only present (or much stronger) on the first image, blue spots are only present (or much stronger) on the second image.

2D gel analysis software

In quantitative proteomics, these tools principally analyze bio-markers by quantifying individual proteins, and demonstrating the partitioning between one or more protein "spots" on a scanned image of a 2-DE gel. Moreover, these tools match spots between gels of similar samples to show, for instance, proteomic differences

between early and advanced stages of an illness. Software packages contain Delta2D, Image Master, Melanie, PDQuest, Progenesis and REDFIN - among others. Whereas this technology is extensively used, the intelligence has not been perfected. For instance, whereas PDQuest and Progenesis have a tendency to agree on the quantification and analysis of well-defined well-separated protein spots, they deliver different outcomes and analysis tendencies with less-defined less-separated spots.

Source: http://en.wikipedia.org/wiki/Two-dimensional_gel_electrophoresis

---

8th International ENT Conference Cairo University

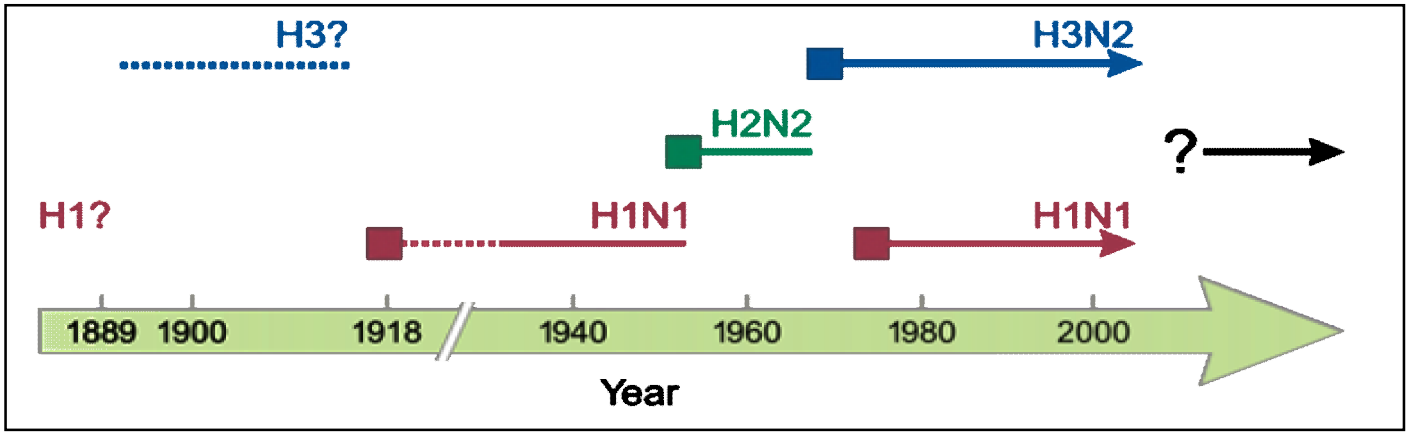
21 to 24 October 2009 , El Suez, Egypt

It is a pleasure to welcome you to the 8th International Conference of the ENT Department, Cairo University. The Scientific program continues the tradition of our previous meetings which have been held in the past. It is organized by COB Conference Oragnizing Bureau , for latest details check the event website.

For more information, please kindly see: http://www.entcairo.com

Swine Flu, a worldwide warning

Every year with the beginning of autumn, a virus called "flu" begins to spread throughout the world. Unfortunately in 2009, another various type of flue started out to disseminate in the world and up to now has killed many people all over the world. One of the characteristics of flu viruses is that they change their surface antigen ligands repetitively and so make treatment difficult in order to control it. Dissimilar to seasonal flu, as people's bodies are somewhat ready to battle the virus, swine flu is a new infection none of us has met before.



This figure depicts how the various strains of influenza have infected the human population in the 20th century.

In addition to observing hygiene considerations, totally, there are two therapeutic approaches against viruses like swine flu consisting Producing antibody and vaccine. Antibodies can also be used in diagnostic tests. For example, Emory university researchers are exploiting a novel and quick method of rapidly manufacturing monoclonal antibody targeted swine flu (H1N1) as diagnostic tests as well as impermanent therapy to hinder infectious disease. The researchers could produce such high affinity monoclonal antibodies against H1N1 by vaccinating volunteers and then after one month, their blood sample was taken and their monoclonal antibodies were optimized to possess a high efficient function. This technique is only a provisional solution to prevent people from swine flu. These human monoclonal antibodies are obtained from blood sample and with using monoclonal antibody technology we are able to reproduce them in a fast and less unmanageable method. On the other hand the researchers at the hospital and clinical senior lecturer at the University of Leicester, infectious department are working on H1N1 to produce vaccine. They are carrying their research on their developed vaccine to evaluate it and the Britain's First Swine-flu Trials is Under Way. The researchers of this center are using 175 volunteers that have been administered this vaccine and their blood is taken to assess how much immunity is produced against such vaccine. This trial begun in July and results are expected to be release in September. The aim of this study is to realize how many doses and what type of vaccine is required to give protection. The early results show that two doses of the vaccine will be needed. If we would like to induce a good immunity in body, it is essential to give priming and then a boosting dose. So it is obvious that every lower dose, the more population could be covered with this vaccine. This trial will also clarify how far apart is required between priming and boosting dose. But the researchers from three another centers comprising Columbia University (Center for Infection and Immunity), Argentina's National Institute of Infectious Diseases, and Roche 454 Life Sciences have decided to decode the whole genomic sequences of influenza pandemic (H1N1) 2009 virus from patients undergoing severe respiratory disease. The researchers are

comparing the current H1N1 virus as an outburst in Argentina with other similar viruses to find out the reason why such virus has created such high mortality. Colombia University has decided to isolate about 150 virus specimen form nasopharyngeal patients and then decode their sequences. Such sequence database will bring many advantages to scientists because they will distinct between sever and mild types as well as how this virus has evolved over time. The researchers are working in Argentina because this country has had the most mortality (165) up to now except U.S. scientists know that every considerable changes in this virus should not be ignored , as it has a significant role in an efficient development of vaccine, and drug. Roche Holding AG's 454 Life Sciences unit, which possess genetic-sequencing technology, is assisting to decode the viruses. With development of vaccines, it should be considered that the patients undergoing autoimmune diseases are more predisposed to flu virus because of two reasons. First, in such patients, the immune system is compromised and the latter that they are taking immunosuppressive drugs that more disturb their immune system. For example in patients suffering systemic lupus erythematosus (SLE), it is better to receive annual flu shots, which are safe and do not increase disease activity. In patients with SLE, both humeral and cellular responses are responsible against flu virus. But in such patients the role of humeral response is deteriorated and we don't know how much the cellular response is involved. For this reason the scientists in University of Groningen in The Netherlands did a research to find out the real fact. They had 54 SLE patients and 54 healthy as control who received subunit flu vaccine. Both groups were vaccinated and after 4 months their blood was taken for evaluation. It became clear that cellular immune especially CD4+ and CD8+ T-cells which are part of the immune response are less induced by vaccination. CD4+ and CD8+ T-cell responses to staphylococcal enterotoxin B (SEB), which was used as a positive control, were normal in patients with SLE, indicating that their decreased cell-mediated response to the flu vaccine was not attributable to a decreased responsiveness of T cells in general. However, it should be bear in mind that taking immunosuppressive drugs like prednisone and/or azathioprine in such patients has a significant role in reducing cellular immune response. In previous studies it was shown that antibody response is diminished and was also confirmed in this study. The scientist tried to create a correlation between humeral and cellular response in SLE patients because CD4+ T helper cells are required for antibody response. But such connection was not obtained through different methods like flow cytometry or ELISpot assay. The authors postulate that the diminished cell-mediated immune and antibody responses to flu vaccination in SLE patients are indicative of what happens in daily practice. "Clinicians should be aware that this combined defect might augment the morbidity and mortality due to influenza virus infection, especially in patients receiving prednisone and/or azathioprine. The last aspect that we would like to point out is that which group of people are more prone to seasonal or swine flu. Centers for disease control (CDC) recommend that, the most predisposed people are those younger than 5 years old, adults 65 years of age and older, pregnant women, and those suffering from pulmonary, cardiovascular and other disorders. But a study at Yale School of Public Health differs from current vaccination recommendations of the Centers for Disease Control (CDC) and the Advisory Committee on Immunization Practices (ACIP). In this study, it is shown that high risk people are different from those who can transmit virus to others in higher proportion. So targeting such group will result in fewer infections and improved survival rates. One of the researchers in Yale University could measure outcomes based on deaths, years of life lost and economic costs by mathematical models. Surprisingly, he found out that school children and their parents are the best targets for vaccinations because school children could transmit virus among other classmates and their parents are the closest persons in contact with them who are responsible for transmitting the virus as well. Therefore these two groups were targeted and were shown that the other parts of population are better protected. The CDC expanded its seasonal flu vaccination recommendations in 2008 to contain children up to 18 years old. Still, Galvani who lead this study in Yale university suggested that preceding, and new, guidelines for both swine and seasonal flu

performed substantially worse than the optimal strategies that she and her group identified. In a comparison which was done in this study. It is completely obvious that the rate of infection, morbidity and mortality and cost will dramatically decrease by changing the targets for vaccination. According to WHO some of the countries in the southern hemisphere, like Chile, Argentina, New Zealand, and Australia seems to be in standard level of influenza prevalence and have passed their peak of influenza activity whereas some countries like South Africa and Bolivia are hitting the highest point in the levels of influenza activity. This report expresses that tropical regions located in Central America and Asia are still at highest peak of influenza activity. Although in the northern hemisphere the areas like North America, Europe, and Central Asia which are considered as the moderate areas the rate of activity is low With some countries experiencing localized outbreaks. Except about some sporadic report about resistant cases to osetamiver, but the antiviral susceptibility tests shows that H1N1 virus is still sensitive to this drug. In over all, if we would like to have a glance at the rate of influenza activity in the world, the following table shows in summery prepared by WHO.

Region	**Cumulative total**	
	As of 13 Aug 2009	
	Cases	*deaths*
WHO regional office for Africa (AFRO)	1469	3
WHO regional office for Americas (AMRO)	105882	1579
WHO regional office for eastern Mediterranean (EMRO)	2532	8
WHO regional office for Europe(EURO)	Over 32000	53
WHO regional office for Asia (SEARO)	13172	106
WHO regional office for western pacific (WPRO)	27111	50
total	Over 182166	1799

This table shows the affected areas and rate of deaths in every area as of 13 Aug 2009. WHO points out that there is no required that countries send more data about this virus because the Above table data is enough to estimate the total cases of every area.

Sources:

1- Jan Medlock ,Alison P. Galvani. Optimizing Influenza Vaccine Distribution. *Science* (2009).

2- Albert Holvast, Sander van Assen, Aalzen de Haan, Anke Huckriede, Cornelis A. Benne, Johanna Westra, Abraham Palache, Jan Wilschut, Cees G.M. Kallenberg, Marc Bijl. Studies of Cell-Mediated Immune Responses to Influenza Vaccination in Systemic Lupus Erythematosus. *Arthritis & Rheumatism*(2009). Vol. 60 (8): 2438-2447.

3- Britain's first swine-flu trials under way, Research led by University of Leicester and University Hospitals of Leicester NHS Trust(2009).

4-News Release: Faculty Experts, Research(2009).

5-http://www.mailmanschool.org/news/display.asp?id=773

6- http://en.wikipedia.org/wiki/H1N1

7- http://www.who.int/csr/don/2009_08_21/en/index.html

Unpredicted and unwanted effects arisen from monoclonal antibodies

Today recombinant proteins are growing every more in treatment of many diseases especially refractory ones like cancers, autoimmune diseases. Among these therapeutic proteins, monoclonal antibodies play a considerable role in treatment of cancers and are introduced in cancer guidelines as the main strategy to control cancer and other resistant diseases. Even though the rate of development and success in monoclonal antibody filed is undeniable, but in recent years, news about side effects after post marketing urged FDA authorities to for companies to recall or add warnings on their drug labels. Even though we know that the main side effects arisen from therapeutic proteins is related to their immunogenicity if they are originate from non-human, here we bring some of the unpredicted and important side effects that has been reported by FDA or the biotechnological Companies.

1- RAPTIVA (efalizumab) was a humanized monoclonal anibody produced by EMD Serono Canada Inc. this drug was approved in 2005 for treatment of patients undergoing moderate to severe chronic plaque psoriasi(18 years or older) who are candidates for systemic therapy or phototherapy. But This Company in 2009 decided to suspend RAPTIVA from the Canadian marketplace due to safety Concerns. A benefit/risk analysis conducted in Europe by the European Medicines Agency (EMEA) Has determined that the benefit/risk in the approved indication for RAPTIVA has become Unfavorable following safety concerns. Three virological confirmed cases and one suspected case of progressive multifocal leukoencephalopathy (PML) have been reported in patients with chronic plaque psoriasis who had been continuously treated with RAPTIVA for three or more years. In addition to PML RAPTIVA is associated with other serious side effects including Guillain-Barré and Miller-Fisher syndromes, encephalitis, encephalopathy, meningitis, sepsis and opportunistic Infections. As a result, the EMEA has determined that the benefits of RAPTIVA no longer Outweigh its risks and has recommended suspension of marketing authorization of RAPTIVA in Europe. EMD Serono Canada Inc. will suspend RAPTIVA from the Canadian marketplace. Prescribers in Canada are advised not to issue any new prescriptions for RAPTIVA and should review the treatment of patients currently taking this medicine to assess the most appropriate alternatives as soon as possible. Abrupt discontinuation of RAPTIVA without substitution treatment may be followed by recurrence of psoriasis or emergence of new psoriasis morphologies, including erythrodermic and pustular psoriasis. Management of patients discontinuing RAPTIVA should include close observation. In case of disease recurrence, the treating physician should institute the most appropriate psoriasis treatment as necessary.

2- Genmab as one of the big companies in research and producing therapeutic monoclonal antibodies had a good antibody candidate for treatment of blood malignancies. Rituximab was before approved in 1997 for treatment of B cell non-Hodgkin lymphoma resistant to other chemotherapy regimens. Rituximab, in combination with CHOP chemotherapy, is now standard therapy in the initial treatment of diffuse large B cell lymphoma and many other B cell lymphomas. Since rituximab was success in treatment, genmab decided to produce a human monoclonal antibody which targets a different region on the CD20 molecule than the current therapy standard rituximab. The new drug called oftatumumab (Arzerra) was supposed to be better the chimeric partner (rituximab) as it was derived from human and less immunogenicity would be expected. On January 30, 2009, Genmab submitted a Biologics License Application to the FDA to use Ofatumumab to treat

cases of CLL. On February 26, 2009, the European Medicine Agency (EMEA) approved a Marketing Authorization Application for the same use. This makes Ofatumumab the first marketing application for an antibody produced by Genmab, as well as the first human monoclonal antibody which targets the CD20 molecule that will be available for patients with refractory CLL. But the last report on 19/8/2009 shows only 11% of 116 rituximab-refractory patients responded to the highest (1 mg) dose of oftatumumab leading to progression-free survival of 6 months. An application for US market approval for Arzerra to treat refractory chronic lymphocytic leukemia (CLL) is under review by the US Food and Drug Administration.

3- TNF α blockers are a group of therapeutic proteins that are attracting interest every day due to their potential usage in autoimmune diseases. But their role in Rheumatoid arthritis is noteworthy. But now this question arise whether arthritis patients should fear the side effects of Enbrel, Remicade, and Humira? The answer is that The TNF (tumor necrosis inhibitor) blockers are considered biological DMARDS. They have a very good benefit/risk ratio. In other words, while potentially serious side effects may develop, they are not common and up 70% of patients may see significant clinical benefit. The class of drugs has been studied in patients for almost 9 years and have been on the market for over 6 years. Two major concerns with these drugs include the risk of serious infection or Lymphoma now. Rheumatoid arthritis patients have an increased risk of developing these two problems compared to the general population. In fact, patients who have the most severe cases of RA are more likely to develop lymphoma than those with milder disease, so it will be of interest to see over time whether treatment with these medications to control disease activity may actually decrease the incidence of this problem. There is evidence that the drugs increase the risk of developing unusual infections like tuberculosis (it is recommended that all patients be screened with a skin test to check for prior exposure to TB before beginning therapy with a TNF blocker). Other serious infections have also been reported since the drug has been on the market. Be extra cautious about taking these medications if you are an insulin dependent diabetic or have another reason to be at increased risk for infection, recurring infections or open wounds/sores. Many rheumatoid patients may do very well on the traditional DMARDS such as Plaquenil (hydroxychloroquine), Azulfidine (sulfasalazine) or Rheumatrex (methotrexate) and may never need to take a TNF blocker. In patients who have significant disease and have reasons they should not take methotrexate (eg. will not avoid alcohol), TNF blockers can be used as a first line treatment.

What we can conclude from the above news is that even though a lot of experimental assays and test is done on monoclonal antibody in order to obtain optimal safety profile specially in terms of immunogenicity , but one point should be considered in mind that every antibody block a specific type of antigens over expressed in abnormal conditions, they also would be able to block normal antigens on healthy tissues and create unwanted effects that sometimes theses side effects are not disclosed in clinical trials and are appeared after post marketing.

Sources:

1- A. Korman et al. , Progressive Multifocal Leukoencephalopathy, Efalizumab, and Immunosuppression : *Arch Dermatol*(2009). Vol. 145(8): 937-942.

2-http://www.eurobiotechnews.eu/service/start-page/top-news

3-http://arthritis.about.com/od/brms/f/fearsideeffects.htm

4-http://en.wikipedia.org/wiki/Ofatumumab

Cover Picture

Title: Immunoflourescence image of the eukaryotic cytoskeleton. Actin filaments are shown in red, microtubules in green, and the nuclei in blue.
Description: In research, antibodies are utilized in various applications. They are most frequently used to recognize and locate intracellular and extracellular proteins. Antibodies are used in flow cytometry to discriminate cell types by the proteins they express; diverse types of cell express different combinations of group of segregation molecules on their surface, and fabricate different intracellular and secretable proteins. They are also used in immunoprecipitation to split proteins and anything bound to them (co-immunoprecipitation) from other molecules in a cell lysate, in Western blot analyses to recognize proteins separated by electrophoresis, and in immunohistochemistry or immunofluorescence to scan protein expression in tissue segments or to locate proteins within cells with the aid of a microscope, Proteins can also be distinguished and quantified with antibodies, using ELISA and ELISPOT methods.
Source: en.wikipedia.org/wiki/Antibody

Title: Human chromosomes (grey) capped by telomeres (white)
Description: A telomere is a area of repetitive DNA at the ends of chromosomes, which keeps the end of the chromosome from damage. During cell division, the enzymes that reproduce the chromosome and its DNA cannot keep on their duplication all the way to the end of the chromosome. If cells divided without telomeres, they would mislay the end of their chromosomes, and the required information it encloses. The telomeres are disposable buffers blocking the ends of the chromosomes and are polished off during cell division and refilled by an enzyme, the telomerase reverse transcriptase. The telomere reduction mechanism in general restricts cells to a fixed number of divisions. Telomeres shield a cell's chromosomes from combining with each other or rearranging - abnormalities which can lead to cancer - and so cells are usually destroyed when their telomeres are consumed. Most cancers are the result of "enduring" cells which have ways of escaping this involuntary destruction.
Source: en.wikipedia.org/wiki/Telomere

Title: Myeloid sarcoma myeloperoxidase
Description: A myeloid sarcoma (chloroma, granulocytic sarcoma, extramedullary myeloid tumor), is a solid tumor composed of immature white blood cells called myeloblasts. A chloroma is an extramedullary manifestation of acute myeloid leukemia; in other words, it is a solid collection of leukemic cells occurring outside of the bone marrow. The condition now known as chloroma was first described by the British physician A. Burns in 1811, although the term *chloroma* did not appear until 1853. This name is derived from the Greek word *chloros* (green), as these tumors often have a green tint due to the presence of myeloperoxidase.
Source: en.wikipedia.org/wiki/Sarcoma,_granulocytic