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Quest

Editorial

"Necessity is the mother of invention" As the saying goes this quest magazine in current issue gives the glimpses of it which has shown in article vaccines. which are given to the individual for the protection against deadly disease causing organisms. Diseases with hideous and scarring effects have become obsolete in first-world countries (and some like polio are also nearing elimination) due to regular vaccination. There are more benefits than risks provided by vaccines. Similarly the organisms are also turning smart by changing their molecular organization in such way that they have developed the resistance to many drugs used for the treatment and this resistance to the drug will be developed by the organism for its survival so in the course of evolution this process of acquiring resistance is going to be never ending process. So time demand the entire process of infection to be mapped so as counter act the spread of infection from the starting point itself, which can be seen in the article new targets for combating malaria. Malaria is an infection caused by single -celled parasites that enter the blood through the bite of an Anopheles mosquito. People who have lived all their life in a country with a high rate of malaria have typically been exposed to malaria parasites many times. After the first exposure, your immune system begins to protect you, so re-infection may cause few or no symptoms. Your immune system does not remain active against malaria for more than a few years if you are not exposed again. This explains why people can live for years in the tropics without being bothered by malaria. However, people from the tropics who spend several years in another country may lose their immune protection. People who have never had a malaria infection (such as young children and travelers) and pregnant women are more likely to have severe symptoms from malaria. Malaria is one of the major causes of preventable death in the world today. It affects more than 500 million people worldwide and causes 1 to 2 million deaths every year. Every effort needs to be made to contain their spread while at the same time pushing forward with the development of effective alternative treatments that are almost certainly going to be needed in the future.

We invite you to read this month's articles and contribute to these discussions. Also, check us out on Facebook and leave us your opinions.

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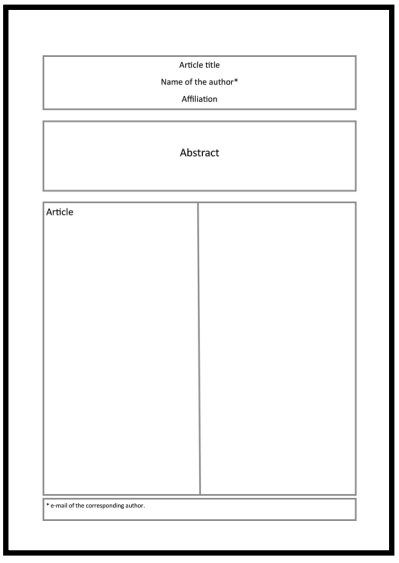
NEWS AND VIEWS:-

REVIEW ARTICLE:-

Notice to Authors

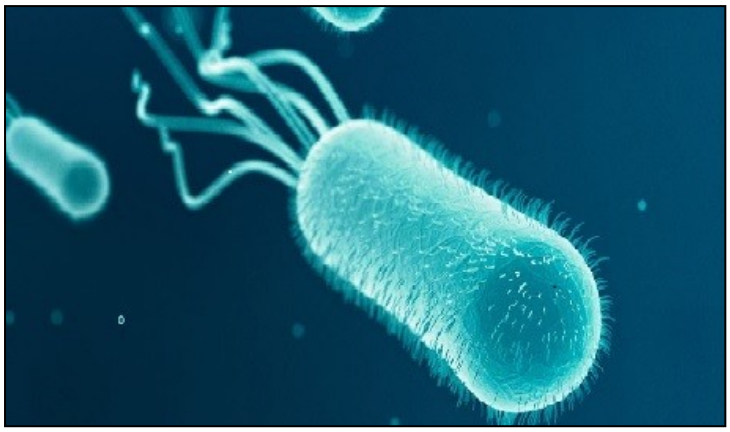
Manuscripts submitted to Quest should adhere to below mentioned criteria.
Research News: About 400 words (1 page)
Research Article: About 2000 words (4 pages)

Common for all: -
Font: Calibri
Font Size: 14
Columns: 2
Line Spacing: 1
Margin: Narrow
References: 1) In text citing, S No, Superscript.
2) Author's name (s), *Journal name*, **Volume No**, Page No**,** (year).
3) Maximum number of references should not exceed than 25.



Bacterial aided water Flow:

"With added microscopic cells, fluids flow as with no obvious resistance"



E.coli, illustrated here, uses their tail-like flagellator swim. A new study reveals that the bacteria' synchronized swimming can eliminate a liquid's resistance to flow**.**

A new experiment finds that water flows best when it's intercalated with synchronized-swimming bacteria.

When we see it may appears that water flows easily. After all, a stream of water flows a lot faster than a stream of other liquids like Castor oil and honey. But water doesn't flow nearly as fast as liquid helium. Such a frosty liquid flows with almost no resistance. Indeed, it is said to have zero viscosity. (Viscosity is a measure of a fluid's resistance to stress. It corresponds to the idea of how "thick" a liquid is.)

But now, by cajoling billions of cells to work together, researchers have made a small sample of a bacteria-laden water solution show no resistance to flow.

"The results are pretty cogent," says Raymond Goldstein a physicist at the University of Cambridge in England. He demonstrated a new study revealing that the motion of microbes can drive the large-scale behavior of liquids.

The new finding reviewed by Physicists Héctor Matías López and Harold Auradou at Paris-Sud University and their colleagues authored the new paper regarding bacterial aided fluid flow in the July 10 *Physical Review Letters.*

They took a small cup filled with water, nutrients and *E. coli* bacteria. There were enough nutrients to fuel the swimming of bacteria, but not enough energy to allow the microbes to divide. Then the physicists dipped a cylindrical probe into the cup. They slowly rotated the cup and measured the force of the twist, or *torque ("*A force that produces rotation, twisting or turning"), exerted by the solution on the probe.

A viscous fluid like honey would tug on and spin the probe. Water also would tug on the probe, just not as much. When infused with a strain of very active *E. coli*, the water solution exerted no torque on the probe. That indicates zero viscosity. In some trials, the viscosity actually became negative: The cup rotated counterclockwise. But the solution exerted a clockwise torque on the probe.

Before the cup spun, the bacteria had been swimming about randomly, Auradou says. But theoretical studies suggest that once the liquid starts to flow, the *E. coli* coordinate their motion. As these rod-shaped bacteria swim, they push water in front and behind themselves. Liquid fills in from the sides, which nudges neighboring bacteria closer together and causes them to line up and swim in a similar direction. The bacteria's collective

pushing increases the speed at which adjacent layers of water can rush past each other. That gives the solution a more efficient and less viscous flow.

This new finding may be especially useful in the lab. Tiny amounts of fluid can be difficult to analyze because samples can get stuck in micro-size passageways. Bacteria may help by ensuring that scientists can measure every last drop. It is also useful in draining water from separated compounds rather using oven to dry water. This can also be beneficial in drip-irrigation system to let water flow till longer distances by making use of environmental *E-coli*, etc.

Source:
student.societyforscienc.org by Andrew Grant August 5, 2015.

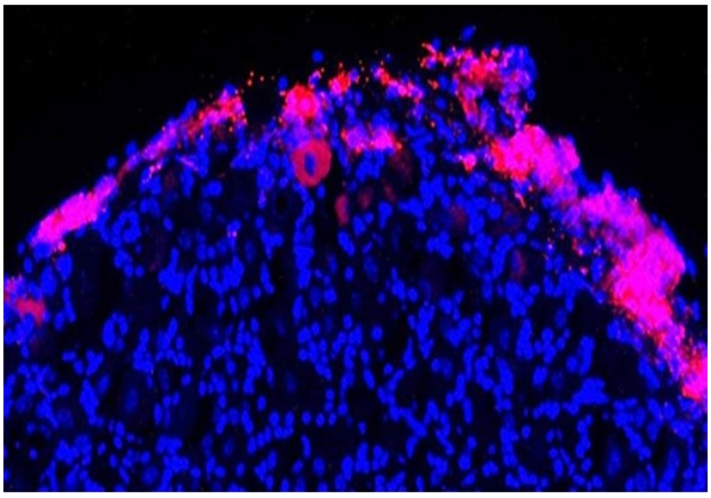
-Contributed by Ruby Kharwar
Ph. D Student

Injected cells acting as drugstores and pain reliefs:

Scientists explain why a treatment for mice with nerve damage works

Nerve damages cause the chronic and worst kind of pain. It can last a long time. No much treatment available for such pain. But there is a type of treatment available, showing its action on mice. And now scientists believe that they have understood how it works.

For the treatment what scientists have done is, they extracted cells from bone marrow of mice and are then injected into the spinal cord of a mouse with nerve damage. Upon — The image shows Cells extracted from bone



marrow (pink) can deliver pain-relieving proteins to nerve cells in the spine (blue).

injection the cells flocked to injured cells where they make a pain-relieving protein. Researchers shared their new finding in the *Journal of Clinical Investigation*. They suspect it might one day lead to improved treatments for people who suffer from chronic pain.

Now researchers know that the marrow cells can relieve pain. But Ru-Rong Ji a neurobiologist stated that the researchers did not know why and how it happened in mice. And it was later explained by his team that the injected cells homed in on their ultimate destination by following chemical signals released by the injured nerve cells. There, the injected cells produced an anti-inflammatory protein called as transforming growth factor beta 1, or TGFB1. That protein was what brought long-term relief. Arnold Caplan, a biologist at Case Western Reserve University in Cleveland, Ohio noted that the injected cells "make drugs at sites of injury", means acts as "drugstores". The cells relieved pain in mice in less than one day. Their effect lasted for more

than a month even if the treatment is given 21 days later of the nerves injury.

What works for mice may not work in people, notes John Farrar. He studies treatments for pain in Philadelphia at the University of Pennsylvania. But Arnold Caplan said that the new data could be used as the basis for a trial in people using human cells. Cells injected into the spinal cord are protected against immune attacks.

Neurobiologist, Ru-Rong Ji believes that people might therefore be able to receive cells from unrelated donors, or even different species. Normally, a person's immune system would reject such an injection of foreign cells and might even be encouraged to make more TGFB1.

Indeed, other studies indicate that the drugstore cells make other pain-relieving compounds in addition to this particular protein, Arnold Caplan pointed out.

According to John Farrar future research must be done to see if these treatments have side effects. TGFB1 is associated with cell growth. So losing control of this protein could cause injected cells to grow out of control. A person treated with them might then develop cancer. But according to Ru-Rong Ji cancer seems to pose only a small risk in this study as the injected cells never became part of the treated spinal tissue. In fact, they disappeared from the spine completely within three months. "A lot more work needs to be done to understand what the long-term downsides would be," Farrar said and the study is promising. "It's very exciting that we should find a set of cells that we could inject that might make a difference."

If external cells can be injected in humans to cure chronic pain then it may lead to find new treatment for pains of different kind like the pain occur during chemotherapy, etc. There's no way to reverse Spinal cord injuries but nerve cell regeneration or improvement of the cell function can help to cure it, so we can even try to check if injected cells can help improving the nerve cell functioning along with pain relieving. If the cells acts in human as the way they showed their effect on mice then many health issues can be solved.

Source: student.societyforscienc.org by Sarah Schwartz July 29, 2015.

-Contributed by Mudit Mayank, M.Sc IGBT Sem-I And Ruby Kharwa

New Targets to combat malaria: invasion specific surface proteins

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Abstract: Malaria is still a life threatening disease present worldwide especially third world population is at the greatest risk. Development of resistance in the malaria parasite is posse's great challenge for development of new drugs against new target candidates. In life cycle of parasite, invasion is the most important pathogenic step. Further propagation will be stopped if any drug or antibody can act at this stage. Two main invasion specific surface proteins PfRH5 and AMA-1 are reviewed.

Causative agents of Malaria

Malaria causing parasite, *Plasmodium* spp. belongs to the Apicomplexa phylum which is well characterized and having invasion specific organs. Out of more than 20 well documented species of Plasmodium*, P. falciparum, P. vivax, P. malariae, P. ovale* infect the humans[1]. These four differ in morphology, immunology and geographical distribution. Among them*, P. falciparum* is showing highest morbidity and mortality worldwide*.* While *P. ovale* is only found in West Africa and *P. malariae* is present worldwide, but with relatively low frequency. The most widespread malaria parasite is *P. vivax* but its infections is rarely fatal[2]. *P. falciparum* (50%) and *P. vivax* (50%) are major causing species in India.

Female *Anopheles* mosquitoes are transmitting vector of malaria. There are about 400 different species of it, but only 30 of these are vectors of major importance[3]. Among them *An. gambiae* is highly prevalent vector in sub-Saharan Africa. In India, *An. culicifacies, An. fluviatilis, An. stephensi, etc.* are found[4].

Symptoms and Diagnosis

Symptoms of malaria appear in 10-16 days after infectious mosquito bit. Preliminary symptom of malaria is fever which is due to the schizont rupture and destruction of erythrocytes. Fever can be intermittent with or without periodicity or continuous which is often accompanied by headache, myalgia, arthralgia, anorexia, nausea and vomiting. Many cases have chills and rigors too. Sometimes symptoms can be non-specific and mimic other diseases like viral infections, enteric fever etc.[5]. The major complications of severe malaria include cerebral malaria, pulmonary edema, acute renal failure, severe anemia, and/or bleeding. Acidosis and hypoglycemia are the most common metabolic complications[6].

Prompt and accurate diagnosis is critical to the effective management of malaria. All clinically suspected malaria is investigated by different microscopy and/or Rapid Diagnostic Test (RDT) methods[7].

Treatment of malaria

Malaria is usually over-diagnosed on the basis of symptoms alone, especially in endemic areas like India, China because of this non-specificity of symptomatology. Around two

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million laboratories confirmed cases of malaria annually. Out of all cases, 40-50% is of *P. falciparum.* It is observed that *P. falciparum* infection may lead to complications in 0.5% to 2% of cases. It leads to the death in about 30% of such cases if timely treatment is not given.

Treatment of malaria is depending upon the factors like types of infections, severity of infection, and status of host, associated condition/ disease. Antimalarial drugs are classified according to their antimalarial activity like Tissue schizonticides, Blood schizonticides, Gametocytocides, Sporontocides and their structure[8]. Malaria mainly treated with chloroquine, primaquine, quinine, quinoline, folate inhibitor, artemisinin drugs and their derivatives. Generally 600mg dosage (for adult) of chloroquine is required for "uncomplicated or low risk malaria" and different dosage of chloroquine and primaquine are used for "complicated or high risk malaria". This days very effective treatment Artimisinin based combination therapy (ACT) is used. Artimisinin is derived from Chinese plant and it produce rapid clearance of parasitaemia and rapid resolution of symptoms is reducing parasite numbers 100 to 1000 folds per asexual cycle and which is much higher than other compounds[9]. Different derivatives of artimisinin(artesunate, artemether, artemotil, dihydroartemisinin) are combined with long term effective drugs (amodiaquine, lumefantrine, mefloquine or sulfadoxine-pyrimethamine).

Life cycle of malaria parasite

The life cycle of malaria parasites is extremely complex and requires specific proteins expression for completion of cycle in both the invertebrate and vertebrate hosts[2]. Genetic complexity, polymorphism and morphological diversity found in malaria parasite, which help it to escape from host immune system. In that allelic variation and different stages specific surface antigens play a major role to misdirect the human immune system.

As shown in figure 1, *Plasmodium* completes their life cycle in two hosts. It involves several stages which begin with the bite of infected female *Anopheles* mosquito. Sporozoites are present in salivary gland of mosquito and they are being transferred into the human body.

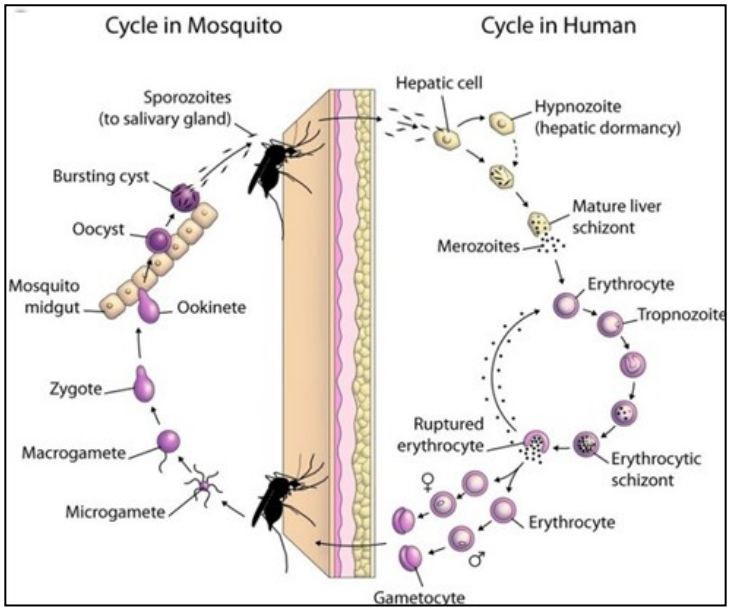

Figure 1 : Life cycle of P. Falciparum (Source: http://ocw.jhsph.edu accession date: 01/04/2014)

Sporozoites enter into the liver and infect hepatic cells which multiply asexually to form schizonts and get mature in liver for 7 to 10 days. This step does not show any symptoms. These schizonts get rupture and releases merozoites. They travel through heart, lung and finally enter into the blood.

invasion. This protein family comprises of five members PfRH1, PfRH2a, PfRH2b, PfRH4, PfRH5 that bind either with sialic acid dependent or sialic acid independent erythrocyte receptors. PfRH3 appears to a transcribed pseudogene[11]. Here figure 3 is showing the signal peptide location, RH family homology region and the trans-membrane region at the C-termini of RH family members.

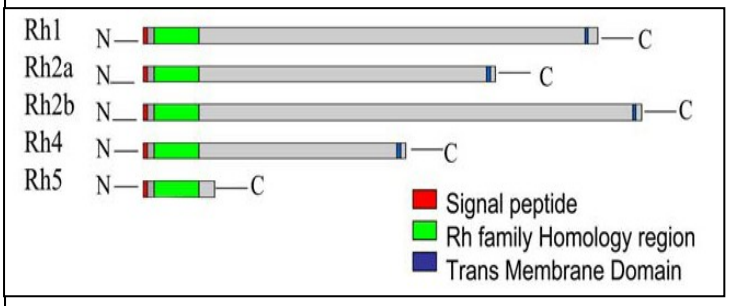

Figure 3 : Schematic representation of RH family with domain architecture in P. falciparum[14]

The overall gene sequence homology between the different RH family members is low, but all contain a number of conserved blocks of amino acids which clearly identify them as members of this ligand family[14]. Apart from these two protein families (involve in secondary interaction), there are also other proteins involve in invasion process like Merozoite Surface Proteins (MSPs), Apical membrane brane Antigen-1 (AMA-1), motor associated protein (TRAP) etc (Figure 4)[13]. From all this surface proteins, highly conserved proteins are always target for the antibodies production or designing of inhibitors by researchers. Here, two main proteins are targeted for invasion inhibitory drug designing. (1) PfRH5 (2) AMA-1

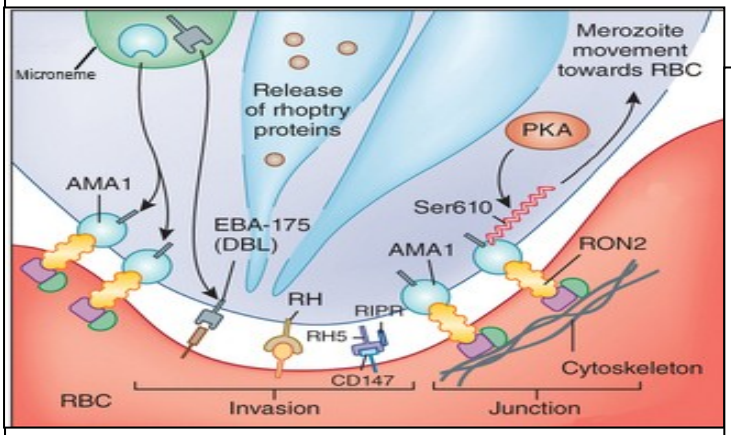

Figure 4 : Different proteins involve in invasion process[13]

PfRH5

P. falciparum Reticulocyte binding like Homologue 5 (PfRH5) is refractory to gene deletion so it plays an essential role in parasite invasion. PfRH5 (PlasmoDB ID: PF3D7_0424100) was first identified by genetic mapping as a key determinant of species specific erythrocyte invasion. Genetic analysis of a *P. falciparum* had mapped the PfRH5 gene on chromosome number 4[18]. The amino acid sequence of PfRH5 ligand is shown in box 1. 143 amino acid sequence at the N-terminus of PfRH5 (from Asn-31-Val-174) is ~22% phylogenetically close with PvRBP1 on the basis of a Clustal alignment. And this region is also shows significant homology with the RBC-binding domain of PfRH4[19].

Box 1 : Amino acid sequence of PfRH5 differs from other member of PfRH family—

MIRIKKKLILTIIYIHLFILNRLSFENAIKKTKN-
QENNLTLLPIKSTEEEKDDIKNGKDIKKEIDNDKENIKTN
NAKDHSTYIKSYLNTNVNDGLKYLFIPSHNSFIKKYSVF
NQINDGMLLNEKNDVKNNEDYKNVDYKNVNFLQYHF
KELSNYNIANSIDILQEKEGHLDFVIIPHYTFLDYYKHLS
YNSIYHKSSTYGKCIAVDAFIKKINETYDKVKSKCNDIK
NDLIATIKKLEHPYDINNKNDDSYRYDISEEIDDKSEETD
DETEEVEDSIQDTDSNHTPSNKKKNDLMNRTFKKMMD
EYNTKKKKLIKCIKNHENDFNKICMDMKNYGTNLFEQL
SCYNNNFCNTNGIRYHYDEYIHKLILSVKSKNLNKDLSD
MTNILQQSELLLTNLNKKMGSYIYIDTIKFIHKEMKHIFN
RIEYHTKIINDKTKIIQDKIKLNIWRTFQKDELLKRILDMS

as it is much smaller around 63kDa (526 amino acids) and it lacks the transmembrane domain at C-terminus. After release from the rhoptries during invasion, PfRH5 forms complex with cysteine-rich antigen named as *P. falciparum* RH5 interacting protein (PfRipr) which facilitates its expression on the merozoite's surface for erythrocyte invasion. In comparison to other merozoite antigens, it shows less genetic diversity. This PfRH5 interact with Ok blood group antigen, basigin (BSG also known as CD147) present on the Red Blood Cells which was being identified by AVEXIS assay (Avidity-based extracellular interaction screen)[15].

Basigin, member of immunoglobulin superfamily (IgSF), is largely composed of extracellular immunoglobulin domains, transmembrane region and cytoplasmic region. Basigin is a highly glycosylated protein which is of 43-66 kDa. It is having 4 isoforms (basigin-1 to 4) caused by alternative transcription initiation and variation in splicing. Basigin-2 (basigin-S/ BSG-S) is a general form having two immunoglobulin domains (N-terminally located D1 domain and a more C-terminally located D2 domain). For interaction with PfRH5, it requires both the domain. As neither of the both domains can individually binds. Extracellular portion of basigin was crystallized in an experiment and its structure was elucidated. This gives the structural classification of domains which are of four types, namely V-sets, C1-sets, C2-sets and I-sets; V-set resembles the domain in the immunoglobulin variable (V) region, C1-set and C2-set resemble the domains in the constant (C) region and I set is intermediate region. D1 domain belongs to the C2-set and D2 domain falls into the category of I-set, can also be regarded as a shortened V-set. There are three potential Asn-glycosylation sites in basigin-2 (BSG-S); one in D1 domain and two in D2 domain shown in (Figure 5) [15].

Interaction of PfRH5 with basigin

From the AVEXIS assay, it was proven that basigin ectodomain reacts with the PfRH5. Deglycosylation or any mutation in glycosylation site does not affect the binding of PfRH5. Different experiments were performed to investigate the basigin-PfRH5 interaction. The invasion by *P. falciparum* of erythrocytes was inhibited by pentamerized basigin ectodomain; strong inhibition was observed at a concentration of 1.0-10µM. Another experiment was performed in which two monoclonal antibodies to basigin strongly inhibited the erythrocyte invasion. One antibody, MEM -M6/6, almost completely block the invasion at the concentration of 10µg/ml. Hence, all the experiments suggest that PfRH5 and basigin both perform as a strong vaccine candidate[16].

PfRh5-act as a vaccine candidate

Pathogenesis of malaria results from a blood stage infection and studies in human and animal models have established that immune response targeting blood stage antigens can protect against malaria or facilitate control of parasitemia. Development of blood stage vaccine against *P. falciparum* has proven extremely challenging task. Different studies suggested that PfRH5 has poor immunogenicity. So it is being immunized in a rat which gives strong antibody response so it is able to inhibit the parasite growth. While in human, antibody responses to this antigen are rapidly lost at the end of high transmission season.

The major constraint of effective antibody me

-diated immunity to the blood stages of *P. falciparum* is the extremely brief window (1 minute) of merozoite exposure to antibodies[17].

AMA-1

Apical Membrane Antigen-1(AMA-1), type-1 integral membrane protein, is also refractory to gene deletion and highly conserved throughout the phylum[13]. Its molecular weight is 83kDa which is going to be proteolytically cleaved at the N-terminal and form mature 66kDa protein. Then it is exported to the merozoite surface[18]. When AMA-1 is uncleaved protein, it characterized by eight intramolecular disulfide bonds and localized in to the apical micronemes. These disulfide bonds form 3 conserved domains. Mature protein is also going to be cleaved at the time of invasion. This is done by membrane bound subtilisin-like protease, PfSUB2 which results in shedding of a 48kDa fragment that will be cytoplasmic transmembrane[19].

AMA-1 (PlasmoDB ID= PF3D7_1133400) is present on chromosome number 11. Box 2 is showing the amino acid sequence which contains 622 residues. Sequence was retrieved from uniprot (ID – Q7KQK5). From 622 amino acids, 10% are polymorphic in nature[20].

MRKLYCVLLLSAFEFTYMINFGRGQNYWEHPYQNSD-
VYRPINEHREHPKEYEYPLHQEHTYQQEDSGEDENTLQ
HAYPIDHEGAEPAPQEQNLFSSIEIVERSNYMGNPWTEY
MAKYDIEEVHGSGIRVDLGEDAEVAGTQYRLPSGKCPV
FGKGIIIENSNTTFLTPVATGNQYLKDGGFAFPPTEPLMS
PMTLDEMRHFYKDNKYVKNLDELTLCSRHAGNMIPDN
DKNSNYKYPAVYDDKDKKCHILYIAAQENNGPRYCNK
DESKRNSMFCFRPAKDISFQNYTYLSKNVVDNWEKVCP
RKNLQNAKFGLWVDGNCEDIPHVNEFPAIDLFECNKLV
FELSASDQPKQYEQHLTDYEKIKEGFKNKNASMIKSAFL
PTGAFKADRYKSHGKGYNWGNYNTETQKCEIFNVKPT
CLINNSSYIATTALSHPIEVENNFPCSLYKDEIMKEIERES
KRIKLNDNDDEGNKKIIAPRIFISDDKDSLKCPCDPEMVS
NSTCRFFVCKCVERRAEVTSNNEVVVKEEYKDEYADIP
EHKPTYDKMKIIIASSAAVAVLATILMVYLYKRKGNAE

Box 2: Amino acid sequence of AMA-1 protein of 3D7 strain From the all 3 domain, Domain I and domain II are highly conserved throughout the Apicoplexa while domain III is less conserved. Same way the polymorphism found more in Domain I and lesser in domain III. Domain I contains several loops which are Ia-If. hydrophobic conserved cleft (region) of domain I is surrounded by the polymorphic residues[20]. Domain I and II share a common core topology called as PAN domain or Apple fold consisting five-strand β-sheet flanked by single helix at one side and three stranded α -sheet at other side[21]. Here figure 5 shows the domain I II III with cysteine disulfide linkage, prosequence which is being cleaved, and transmembrane C-terminal.

Properties of AMA-1 residues

AMA-1 is having polymorphic residues surrounded by the several conserved hydrophobic residues. Hydrophobic cleft is formed by Val169, Leu176, Phe183, Met190, Tyr202, Val208, Met224, Tyr251, Ile252, Met273, Leu357, Phe367[21,22].

And polymorphic residues are Glu197, His200, Phe201, and Asp204 from which Glu197 and His200 are highly polymorphic, Phe201 is less polymorphic and Asp204 is strictly dimorphic. Several other polymorphic sites are there but they do not affect the receptor or antibody binding[21].

AMA-1 and RON complex interaction

During the invasion process, tight junction formation occur between apical end of the merozoite and the host cell which is followed by the movement of the junction adhesion zone (moving junction) towards the posterior pole. Moving junction (MJ) is very different feature of Apicomplexan invasion which was

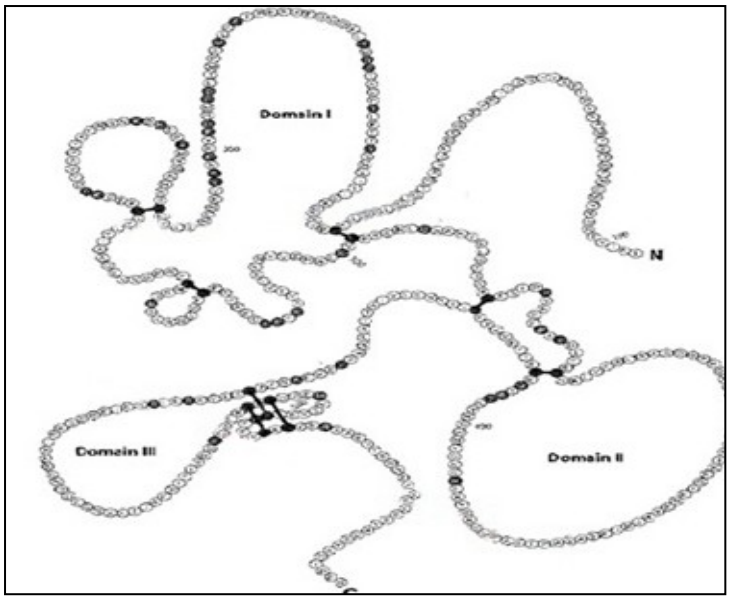

Figure 5 : Schematic structure of domain I II III of AMA-1

first observed in *Plasmodium* spp. in late 1970s[23.]

AMA-1 form complex with RON i.e. Rhoptry Neck Protein releases from the Rhoptry secretary organelle present in parasite. It is inserted into the host plasma membrane (erythrocyte membrane) and form a complex of RON2/4/5/8. Figure 6 is showing the steps of secretion and junction formation of AMA-1 and RON complex[24]. RON protein family of parasite is highly conserved throughout the Apicomplexa. This complex contains RON2 (assumed to contain 3 hydrophobic helices), RON5 (contain only one predicted hydrophobic helix) and RON4, RON8 (both appear to be soluble proteins).

RON2 is only protein which is having transmembrane domain. RON4/5/8 remains inside the host cell and form complex with cytoskeletons. Several models were proposed that AMA-1 interacts with the RON2. So it is very essential for moving junction formation. But along with that, other proteins are also equal important as they are making junction with erythrocyte cytoskeletons[24]. RON2 protein contains 2189 amino acid residues in 3D7 strain and its genes are located on chromosome number 14. The model which says that tight junction composed of AMA-1-RON2 interactions with 1:1 stoichiometry is widely accepted. But apart from this several other evidences and models were proposed that AMA-1 interacts with RON4 or RON5.

AMA-1 as a vaccine candidate

As AMA-1 is attractive candidate for malaria vaccine, its extracellular domains are targeted for the antibodies production. Vaccination against recombinant AMA-1 has been demonstrated to induce protection against different models like rodent, monkey etc. Vaccines against AMA-1 were not shown to be effective in recent studies, probably because of in-

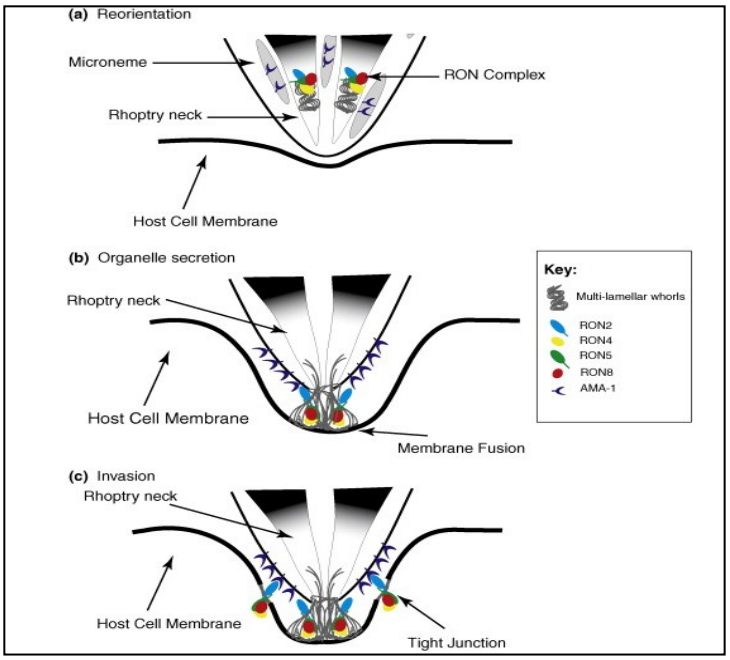

Figure 6 : Secretion and junction formation of AMA-1 and RON complex[24]

sufficient cross-protection against diverse malaria strains or insufficient immunogenicity. FMP2.1/AS02A is a monovalent blood-stage

malaria vaccine based on AMA1 from the 3D7 strain of *P. falciparum*[25]. Conserved regions of AMA-1 need to be understand which were already reported. But AMA-1 diversity is also high and there are regional differences found in different allelic classes. So vaccine development is a very challenging task for researchers.

References:

1. Duraisingh, M. T., Desimone, T., Jennings, C., Refour, P. & Wu, C. in *Mol. Mech. parasite invasion* 46–57 (2008).
2. Tuteja, R. *FEBS J.* **274,** 4670–9 (2007).
3. Butler, D. *Nature* 1–255 (2013). doi:10.1038/nature.2013.13535
4. WMR. *World Malaria Report 2013*. 143 (2013). at <http://www.who.int/malaria/publications/country-profiles/profile_ind_en.pdf>
5. National Institute of Malaria Research. *Malar. India* (2011). at http://www.mrcindia.org/Guidelines for Diagnosis2011.
6. Trampuz, A., Jereb, M., Muzlovic, I. & Prabhu, R. M. *Crit. Care* **7,** 315–23 (2003).
7. Moody, A. *Clin. Microbiol. Rev.* **15,** 66–78 (2002).
8. Bruce-Chwatt, L. J. *Bull. World Health Organ.* **27,** 287–90 (1962).
9. WHO. guideline for the treatment of malaria. *Guidel. Treat. Malar.* **2nd editio,** (2010).
10. Boyle, M. J., Wilson, D. W. & Beeson, J. G. *Int. J. Parasitol.* **43,** 1–10 (2013).
11. Lopaticki, S. *et al. Infect. Immun.* **79,** 1107–1117 (2011).
12. Tham, W.-H., Healer, J. & Cowman, A. F. *Trends Parasitol.* **28,** 23–30 (2012).
13. Cowman, A. F. & Crabb, B. S. *Cell* **124,** 755–66 (2006).
14. Rodriguez, M., Lustigman, S. & Montero. *PLoS One* **3,** e3300 (2008).
15. Crosnier, C. *et al. Nature* **480,** 534–537 (2011).
16. Muramatsu, T. *Expert Opin. Ther. Targets* **16,** 999–1011 (2012).
17. Williams, A. R. *et al. PLoS Pathog.* **8,** 1–15 (2012).
18. Howell, S. a, Withers-Martinez, C., Kocken, C. H., Thomas, a W. & Blackman, M. J. *J. Biol. Chem.* **276,** 31311–20 (2001).
19. Harris, P. K. *et al. PLoS Pathog.* **1,** 241–51 (2005).
20. Healer, J., Triglia, T., Hodder, A. N., Gemmill, A. W. & Cowman, A. F. *Infect. Immun.* **73,** 2444–51 (2005).
21. Coley, A. M. *et al. PLoS Pathog.* **3,** 1308–19 (2007).
21. Henderson, K. A. *et al. Structure* **15,** 1452–66 (2007).
22. Aikawa, M., Miller, L. H., Johnson, J. & Rabbege, J. *J. cell Biol. Biol.* **77,** 72–82 (1978).
24. Proellocks, N. I., Coppel, R. L. & Waller, K. L. *Trends Parasitol.* **26,** 297–304 (2010).
25. Thera, M. A. *et al. N. Engl. J. Med.* **365,** 1004–1013 (2011).

Vaccines: A Glimpse

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Abstract: Development of vaccines has been a huge success story in the modern medicine. It is responsible for huge reduction in morbidity and mortality worldwide. This article is directed towards readers who wish to obtain information about definition of vaccine, history of vaccine, components of vaccine, types and classification of vaccine and how vaccine work.

What is a vaccine[1]?

The word vaccine was originally derived from latin word '*Variolae vaccinae*' which means cowpox. Dr. Edward Jenner of England was the first person to demonstrate in 1798 that cowpox lesions could be used to prevent smallpox in humans. Presently the term 'vaccine' is used to define all biological preparations made from micro-organisms to enhance immunity against diseases. Vaccines can be prophylactic or therapeutic in nature. Vaccines are usually administered in liquid form either orally, by injection or by intranasal route.

Characteristics of an ideal vaccine

Ideal vaccine should provide long lasting immunity against a particular disease. It should induce both cellular and humoral immunity. It should not induce autoimmunity and hypersensitivity. It should be inexpensive to produce, easy to store, transport and administer. It should be generally perceived as safe by people to whom it is to be administered.

Brief history of vaccine development[11, 13, 16]

In 7th century, Buddhist monks of India used to travel long distances on foot across dense forests where they were frequently bitten by poisonous snakes. In order to develop immunity against snake bites they used to drink snake venom. This was one of the first attempts to prevent disease by using the disease–causing organism against.

The practice of variolation developed in Central Asia in the second millennium. Variolation is defined as the practice of inoculating the dried pustules of smallpox (caused by the variolae virus) from a sick individual into a healthy individual so as to develop immunity against disease in the healthy individual. This practice of variolation spread from central Asia to China, Turkey, Africa and other parts of Europe.

Dr. Edward Jenner of England is considered as the first person to vaccinate an individual against smallpox by inoculating him with cowpox virus (closely related to human smallpox virus). The first inoculation was carried out in the year 1798 and it marked the beginning of vaccine era. The term vaccination was derived from vaccine virus. The practice became widely popularized from thereon.

At the end of the 19th century, Louis Pasteur

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applied the concept of vaccination to other diseases. He demonstrated that the harmful nature of disease-causing organisms could be weakened (or attenuated) in the laboratory. He first demonstrated the effectiveness of vaccines against chicken cholera and anthrax in animals, before developing his vaccine against rabies for use in humans in 1885.

In 1886, Daniel Elmer Salmon and Theobald Smith of USA demonstrated that vaccines could be produced not just from live organisms, but also from killed disease-causing organisms. Their discovery led to the subsequent development of inactivated vaccines against several human diseases.

In the early 20th century, it was discovered that some diseases were caused not by bacteria themselves, but by the toxins that they produced. Inactivated toxins acted like vaccines by providing protection against these toxin-induced diseases. These vaccines are now known as toxoids.

By the end of the 20th century, a spurt of innovation led to the development of several new methods of producing vaccines including by recombinant organisms, by conjugation of polysaccharides to carrier proteins, and by the assembly of virus-like particles.

Components of vaccine

Vaccines are made up of ingredients collectively known as excipients. Vaccines are usually formulated by mixing antigens with other fluids such as water or saline, additives or preservatives, and sometimes adjuvants. These excipients make sure that the quality and potency of vaccine is maintained over its shelf life. Vaccines are usually formulated as liquids, but may also be freeze dried and reconstituted just before use.

Preservatives

Preservatives are added to vaccines formulation to ensure sterility of vaccine during its shelf life. The preservatives added are such that they do not change or alter the nature of antigens present in vaccine formulation. They are non-toxic to humans in the concentration used and do not reduce the immunogenicity of the vaccine itself. Some of the commonly used preservatives are phenol, benzethonium chloride, 2-phenoxyethanol and thimerosal.

Adjuvants[12]

Adjuvant is a component that potentiates the immune response to an antigen and/or modulates it towards the desired immune response. In the traditional vaccines impurities or other components of organisms act as adjuvants. For example diphtheria-tetanus- pertussis (DTP) vaccine contains two potent adjuvants from whole cell pertussis vaccine (LPS and PT), whole cell typhoid and cholera vaccines have potent adjuvants (LPS and cholera toxin). Therefore, purified, synthetic vaccines require potent adjuvants. Many potentially protective antigens are weak immunogens. Protein antigens injected in saline typically produce weak and transitory antibody responses while those injected in effective adjuvants produce strong and sustained responses

Properties of an ideal adjuvant[12]

An ideal adjuvant should be safe and the preparation should elicit a protective immune response with weak antigens including polysaccharide-protein conjugates with lower doses of antigens and with fewer injections. It

In blood stage, it invades into the erythrocytes and multiplies to forms 16-32 daughter cells in erythrocytic schizonts. Again it releases merozoites. Some of the infected blood cells leave the cycle of asexual multiplication. They develop into gametocytes which is sexual forms of the parasite.

Again mosquito bites an infected human; it ingests the gametocytes, which develop further into mature sex cells called gametes. The fertilized female gametes develop into actively moving ookinetes that burrow through the mosquito's midgut wall to form oocysts which develop numbers of active sporozoites. Oocyst bursts to release it into the body cavity that travel to mosquito's salivary glands. The cycle of human infection begins again when the mosquito bites another person.

Invasion stage

Erythrocyte invasion, multistep process, is an obligatory step during blood stage. It involves a cascade of molecular events between merozoites and host erythrocytes. Figure 2 shows the merozoite invasion of red blood cells which takes only 60 seconds to invade. It appears to occur in step-wise process. 1. Initial contact occurs between any surface of free merozoite and human red blood cell which include merozoite surface proteins. 2. The merozoite then re-orientate such that the apical end contacts the human red blood cell surface. It is mediated by apical organelle (microneme and rhoptry) proteins. 3. After that merozoite invade using actin-myosin motor. 4. mature invasion is thought to result in the loss of specific surface proteins and leads-to the intra-erythrocytic development cycle[10]. Merozoites release from the erythrocytes every 48hrs and rapidly invade into the new erythrocytes. Invasion of *P. falciparum* merozoites into human erythrocytes involves multiple ligand-receptors interactions[11]. Malaria parasite surface protein act as a ligand which are of two molecular protein families known as EBL (erythrocyte binding like) and RBL (reticulocyte binding like) proteins. This EBL protein family is also known as the duffy binding like (DBL) protein family. In *P. falciparum*, members of these families are termed PfEBL(*P. falciparum* erythrocyte binding-like) and PfRH (*P. falciparum* reticulocyte binding-like homolog) proteins[12].

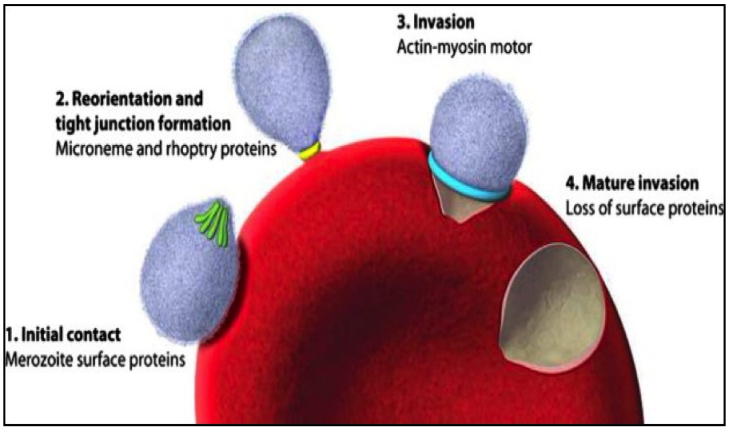

Figure 2 : Merozoite invasion of Red Blood Cells [13]

Erythrocyte binding like (EBL) or duffy binding like (DBL) proteins are localized to the micronemes which includes EBA-175, EBA-140 (also known as BAEBL) and EBA-181 (also known as JSEBL). EBA-175 and EBA-140 form sialic acid dependent interactions to the glycophorins A and C, respectively. The receptor for EBA-181 is still unknown. EBA-165 is thought to be a pseudogene. EBL-1 is not expressed in some parasite lines, as it has missense mutations within the coding region[1,11] *P. falciparum* reticulocyte binding like homologus protein (PfRH) is located in the rh-

optries and key determinants of merozoite should promote an appropriate immune response, namely cellular or antibody immunity depending on requirements for protection. The adjuvant should be stable with regard to adjuvanticity and toxicity without any interaction with the antigen. It should be biodegradable, immunologically inert and cheap to produce.

Uses of adjuvants

Adjuvants have been used with conventional vaccines to elicit early, high and long-lasting immune response. But their role becomes very important especially for purified, synthetic vaccines which are poorly immunogenic. Synthetic and subunit vaccines are expensive to produce. But with the use of adjuvants, less antigen may be required to stimulate the immune response, thus saving cost of vaccines. They are also needed to reduce the amount of antigen or the number of immunizations needed for protective immunity. Adjuvant's significance is also due to its ability to selectively modulate the immune response to elicit humoral and/or cellular immune responses and also uptake of antigens by the mucosa.

Classification on basis of adjuvanticity

Vaccine adjuvants can be grouped into following three types:

1. Causing depot formation at the site of injection - For example, mineral compounds, oil-based adjuvants, liposomes;
2. Acting as delivery vehicles for the antigens which may help in targeting antigens to immune competent cells - For example, liposomes, oil adjuvants;
3. Acting as immunostimulators - For example, Freund's complete adjuvant (FCA), muramyl dipeptide(MDP), lipopolysaccharide (LPS), lipid A, monophosphoryl lipid A (MPL), pertussis toxin (PT), cytokines.

Mechanisms of adjuvant action[2]

Adjuvants exert their immune-enhancing abilities by helping in the translocation of antigens to the lymph nodes where they can be recognized by T cells. Adjuvants provide physical protection to antigens which grants the antigen a prolonged delivery. Adjuvants also help to increase the capacity to cause local reactions at the site of injection, inducing greater release of danger signals by chemokine releasing cells such as helper T cells and mast cells. They are also believed to increase the innate immune response to antigen by interacting with Toll-like receptors (TLRs)on accessory cells.

Types of adjuvants[3, 5, 6, 7, 8]

1. Aluminum-containing adjuvants: E.g.; aluminum phosphate, aluminum hydroxide and alum-precipitated vaccines
2. MF 59:a oil-in-water emulsion
3. Freund's adjuvant:
4. Freund's complete adjuvant (FCA)-Mineral (paraffin) oil mixed with killed Mycobacteria
5. Freund's incomplete adjuvant (FIA)- Water -in-oil emulsion without Mycobacteria
6. Microorganism - derived adjuvants: e.g. muramyl dipeptide (MDP), lipid A, trehalose dimycolate (TDM) etc.
7. ISCOMSs9, 10 are 40 nm large particles made up of saponins (Quil A), lipids, cholesterol and antigen, held together by hydrophobic interactions between the first three components.
8. Liposomes

9. Virosome : e.g. Membrane bound hemagglutinin and neuraminidase derived from influenza
10. Poly(lactide-co-glycolide) microparticles
11. Nucleic acid based adjuvants: e.g. unmethylated CpG dinucleotides.
12. Mucosal adjuvants: e.g. CpG motifs; Imiquimod, Resiquimod, mutants of heat-labile enterotoxin from E.coli , chitosan and carbopol
13. Cytokines: e.g.IL-12

Problems in development of adjuvants[12]

There are numerous problems faced by scientists while developing new adjuvants like limited adjuvanticity. Lack of reliable animal models for many diseases against which vaccines are being developed is another area of concern. Some of the adjuvants show toxicity, therefore, toxicity and adjuvant activity must be balanced to obtain maximum immune stimulation with minimal adverse effects. Some other side effects which are observed are local reactions (such as inflammatory response, local pain, tissue lysis, granulomas and hypersensitivity reactions) and systemic effects.

Types of vaccines[13]

Vaccines may be composed of either the entire disease-causing microorganism or some of its components. They may be constructed in several ways:

1. **Attenuated vaccines**: They are made from living organisms that have been weakened, usually from cultivation under sub-optimal conditions (also called attenuation), or from genetic modification, which has the effect of reducing their ability to cause disease, e.g. Measles, Mumps, Rubella, Varicella zoster etc.
2. **Inactivated vaccines:** They are made from whole organisms that have been inactivated by chemical, thermal or other means, e.g. Hepatitis A, Influenza etc.
3. **Sub-unit Vaccines:** They are made from components of the disease-causing organism, such as specific proteins and polysaccharides, or nucleic acids, e.g. Hepatitis B etc.
4. **Toxoid vaccines:** They are made from inactivated toxins of toxin-producing bacteria, e.g. Tetanus, Diphtheria etc.
5. **Conjugated vaccines:** They are made from the linkage (conjugation) of polysaccharides to proteins, e.g. Pneumococcal, Meningococcal, Haemophilus influenza type b etc.
6. **DNA vaccines:** They are piece of DNA (plasmid) genetically engineered to produce specific antigens.
7. **Recombinant vector:** This vaccine consists of harmless vector which expresses antigens stimulating immune response.

Classification of vaccines

Attenuated and inactivated vaccines are said to belong to **first generation vaccines**. They used whole organisms either live, weakened or killed as vaccines. There was however a small of probability of live attenuated forms reverting to virulent form and causing the disease they were supposed to prevent. This led scientists to develop second generation of vaccines. Sub-unit, toxoid and conjugated vaccines are considered as **second generation vaccines**. These vaccines are made up of protein antigens and recombinant protein components. They are able to generate T helper cell and antibody response but not killer T cell response. The need to have complete immune response against pathogenic organism

led us to develop third generation of vaccines. DNA and recombinant vector vaccines are **third generation vaccines**.

It is absolutely not necessary to administer separate vaccines individually. **Combination vaccines** are available which reduces the number of times a person needs to go for vaccination. There are two types of combination vaccines available. First type combines several serotypes of a disease causing organism in a single vaccine e.g., 13-valent pneumococcal conjugate vaccine. This type of vaccine may contain antigens against several types of the same disease-causing organism, providing protection against each type. Second type combines vaccines against different disease causing organisms and it provides protection against several diseases such as diphtheria, tetanus, pertusis, hepatisis B, polio etc. Combination vaccines may incorporate both viral and bacterial vaccines and may contain toxoids, purified protein sub-unit vaccines, conjugated polysaccharide vaccines, recombinant protein vaccines and inactivated viral vaccines.

How vaccines work?[14, 15]

When inactivated or weakened disease-causing microorganisms enter the body, they initiate an immune response. This response mimics the body's natural response to infection. But unlike disease-causing organisms, vaccines are made of components that have limited ability, or are completely unable, to cause disease. The components of the disease-causing organisms or the vaccine components that trigger the immune response are known as "antigens". These antigens trigger the production of "antibodies" by the immune system. Antibodies bind to corresponding antigens and induce their destruction by other immune cells.

The induced immune response to either a disease-causing organism or to a vaccine configures the body's immune cells to be capable of quickly recognizing, reacting to, and subduing the relevant disease-causing organism. When the body's immune system is subsequently exposed to a same disease-causing organism, the immune system will contain and eliminate the infection before it can cause harm to the body.

The effectiveness and the duration of the protective effect of a vaccine depend both on the nature of the vaccine constituents and on the manner in which they are processed by the immune system. Some disease-causing organisms, such as influenza, change from year to year, requiring annual immunization against new circulating strains.

In very young children, the immune system is immature and less capable of developing memory. In this age group, duration of protection can be very short-lived for polysaccharide antigens.

References:

1. Prakashan, Delhi, India, 2002; pg Swarbrick, J and Swarbrick, J. *Encyclopedia of pharmaceutical technology;* Third edition, volume 6, CRC Press, Taylor and Francis, 2006.
2. Singh M. *Vaccine adjuvants and delivery systems*; A. John Wiley & Sons, Inc. Publication, Hoboken, New Jersey, 2007.
3. Vyas SP, Khar RK. *Controlled drug delivery: concepts and advances*; First edition, Vallabh no: 156-157.

4. Carter SJ. *Cooper and Gunn's Tutorial pharmacy;* Sixth edition, CBS Publishers and Distributors, 2005.
5. Ananthanarayan R and Paniker CKJ. *Text book of Microbiology*; Seventh edition, Orient Blackswan, 2005.
6. Gupta RK. and Siber GR. *Adjuvants for human vaccines - current status, problems and future prospects;* Vaccine, 13(1995), 1263-1276.
7. Hunter RL. *Overview of vaccine adjuvants: present and future*; Vaccine, 20 (2002) S7–S12.
8. Aguilar JC and Rodriguez EG. *Vaccine adjuvants revisited;* Vaccine, 25 (2007) 3752–3762.
9. Martin J. Pearse, Debbie Drane; *ISCOMATRIX adjuvant for antigen delivery;* Advanced Drug Delivery Reviews, 57 (2005) 465– 474.
10. Sjolander A and Cox JC. *Uptake and adjuvant activity of orally delivered saponin and ISCOM vaccines*; Advanced Drug Delivery Reviews, 34 (1998) 321–338.
11. Plotkin SL and Plotkin SA. A short history of vaccination. In Vaccines 5th edition, S Plotkin, W Orenstein and P Offit, Eds, Saunders Elsevier, China, 2008.
12. O'Hagan DT. *New generation vaccine adjuvants*; *In Encyclopedia of life sciences*; John Wiley and Sons Ltd., 2007.
13. www.wikipedia.org
14. www.pharmainfo.net.com
15. www.informaworld.com
16. www.fda.gov


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