ATTACKER'S STRATEGY

Researchers unearth the trick employed by kala azar's deadly germ, writes **G.S. Mudur**

Biologist Amitabha Chattopadhyay has just unearthed a parasite's little secret. It might help medical scientists gain an upper hand over Leishmania donovani, the creature that causes leishmaniasis, or kala azar, and has long tormented eastern India. Chattopadhyay, a senior researcher at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, and collaborating scientists in New Delhi, have gained fresh insights into how the parasite enters human cells — information that might be crucial to block the process and prevent the disease.

The parasite enters the human body through the bite of the sandfly and infects a class of cells called the macrophages, causing persistent infection which left untreated can turn fatal. The creature multiplies inside the macrophages and the infected cells eventually rupture, unleashing more parasites into the bloodstream. Leishmaniasis is marked by anemia, weight loss, fever, and an enlarged spleen. The emergence of parasites resistant to commonly used anti-leishmaniasis drugs has triggered off a search for new ways to tackle the disease.

Chattopadhyay is an expert on the biology of cell membranes, the thin biological structures that make up the surfaces of cells, separating the inside of a cell from the outside and help individual cells maintain their identities. His collaborator is medical biologist Rentala Madhubala, a professor at the school of life sciences at the Jawaharlal Nehru University (JNU), New Delhi. Madhubala has been pursuing *Leishmania donovani* for 14 years. Much of her work has been aimed at devising new vaccines.

PERSISTENT INFECTION: Leishmaniasis victims in Bihar

Last year, she reported successful animal experiments of a vaccine that uses genetic material of the *Leishmania donovani* itself to immunise animals against the parasite, also called a DNA vaccine. But vaccines have traditionally taken a long time to develop. The new study on the parasite's entry mechanism might provide a faster route to tackle the infection for which new drugs are urgently required. Bangladesh, Brazil, India, and Sudan make up 90 per cent of leishmaniasis cases around the world. Studies show that 60 per cent of leishmaniasis in Bihar, the worst-affected state, are resistant to the standard drug called sodium antimony gluconate.

"All existing drugs work after the parasite has already infected macrophages and people are sick," said Chattopadhyay. "The new finding may lead to new ways to prevent the parasite from entering macrophages in the first place," he said, cautioning that more detailed studies will be required to take this ahead into animal trials.

In test-tube experiments carried out at the CCMB and JNU, the researchers have shown that the entry of the parasite hinges on the amount of cholesterol in the membranes of the macrophages. The experiments show that lowering the cholesterol in the macrophage membranes hampers the ability of the parasite to infect the cells.

Although it has long been known that macrophages are the parasite's target cells, the molecular mechanisms of the parasite-macrophage interaction have remained unclear. Over the past decade, several studies have pointed to several proteins on the surface of macrophages that might serve as receptors, or gateways, that allow the parasite to enter into the cells. The multiple receptors reflect what scientists describe as a "redundancy" in the parasite's entry mechanism. It might use any one of these receptors to enter macrophages. But this makes preventing infection difficult because blocking one receptor still leaves open other gateways that the parasite can use to enter the macrophages.

In a report published in this month's issue of the *Molecular and Biochemical Parasitology*, the CCMB and the JNU researchers report that loss of cholesterol from the macrophages results in marked reduction in the extent of leishmaniasis infection. The scientists used macrophages from mice to evaluate the role of cholesterol during the parasite's entry. The other team members were Thomas Pucadyil at the CCMB and Poonam Tewari at JNU.

In typical laboratory dishes teeming with tens of thousands of mice macrophages, the researchers added a chemical called methyl-beta-cyclodextrin, a chemical known to mop up cholesterol from macrophages. When the cholesterol-depleted macrophages were exposed to *Leishmania donovani*, instead of eagerly latching on to their target cells, the parasites' zeal for the macrophages significantly slackened.

The number of the cholesterol-depleted macrophages that became infected with the parasite was less than half the number of infected macrophages with normal levels of cholesterol. The researchers have also shown that this reduction in the host-parasite interaction in the case of the cholesterol-depleted macrophages can be reversed by simply giving back the cholesterol in the macrophages.

The new findings fit in with recent observations that cholesterol within the membrane sometimes forms structures called 'lipid rafts' that serve as gateways for the entry of microorganisms into cells. "They're like tiny lifeboats on the surface of the cell that pick up the parasite from outside the cell and let it pass through the membrane into the interior of the cell," says Madhubala.