Could you explain the significance and prevalence of human parvoviruses? Why are you focusing on parvoviruses, and human parvovirus B19 (B19V) in particular?

Small and highly resistant, parvoviruses are excellent models to optimise strategies aiming to guarantee the elimination of viruses that might potentially contaminate human plasma. The goal is to minimise the residual risk of pathogen transmission to patients.

B19V is typically associated with a mild disease named erythema infectiosum. During pregnancy or in individuals with underlying immune or haematologic disorders, B19V may cause more severe syndromes such as hydrops fetalis, arthopathies and severe cytopaenias.

Your research into parvoviruses has significantly advanced understanding of inactivation mechanisms but also of mechanisms of cell infection. Could you outline the process of discovery which has led to these new insights?

In order to understand how highly resistant viruses, such as parvoviruses, can be inactivated without disturbing the valuable plasma proteins, we follow two strategies. We study the mechanism of inactivation by various physicochemical treatments and also how the robust parvovirus is uncoated inside the cell during the infection process. *In vitro* inactivation and intracellular uncoating show similarities: they are not mediated by capsid disintegration, but by a series of specific structural transitions leading to the release of the virus DNA. The instability of the viral DNA inside the capsid is the weak point of the robust parvovirus and a promising target for inactivation strategies.

Comparison studies also revealed that although DNA release from intact capsids seems to be common among parvoviruses, it occurs much more promptly and to a greater extent in B19V, explaining its lower resistance to inactivation treatments.

You demonstrated that the narrow tissue tropism of B19V is largely determined by the limited expression of a cellular receptor required for virus internalisation. How did you reach this conclusion?

B19V binds to cells through the primary receptor globoside. However, the pathogenesis and tropism of B19V cannot be explained if globoside is the unique receptor. We found that the immunodominant part of the capsid – the VP1 unique region (VP1u) – is not accessible to the exterior of the capsid as is generally thought, but becomes accessible upon receptor binding. This structural change was necessary for the virus to enter the cell, which led us to suggest that VP1u interacts with a cell receptor to allow virus internalisation. The expression of the VP1u interacting receptor is remarkably limited to a few cell types from the erythroid lineage, which fits with the natural tropism of B19V.

By what mechanism is B19V trafficked inside human cells?

Parvoviruses follow a complex route full of obstacles from the cell plasma membrane to the nucleus where they replicate. This implies multiple interactions with cellular proteins and virus structural rearrangements. Although parvoviruses have a similar strategy to infect the cell, there are significant differences. For example, whereas parvoviruses require the acidic conditions inside intracellular vesicles (endosomes) to change in structure, B19V changes in conformation upon receptor binding on the cell surface. Thus, the virus is less dependent on intracellular acidic conditions to infect the cell.

Your paper published in *PLOS Neglected Tropical Diseases* drew a link between malaria and B19V infection. What is the nature of this link?

Severe anaemia is a common and life-threatening complication of malaria in children. B19V coinfection has been identified as a major factor in its pathogenesis, but there are significant regional differences. Unexpectedly high seroprevalence and pathogenic potential of B19V have been observed in certain malaria-endemic countries in parallel with local use of chloroquine (CQ) as a first-line treatment for malaria.

We hypothesised that CQ promotes B19V replication and that, as a consequence, it contributes indirectly to severe anaemia. To investigate this, we studied the effect of CQ and other common antimalarial drugs on B19V infection *in vitro* and the possible epidemiological consequences for children from Papua New Guinea. Our laboratory and clinical data strongly suggest that CQ and its derivatives exacerbate B19V-associated severe anaemia by promoting B19V replication.

How does CQ boost B19V infection? How will the impact of this discovery change health policy surrounding its use?

Apart from its antimalarial effects, CQ inhibits endosomal acidification, which is required by many viruses to infect the cell. CQ inhibits parvovirus infections because it blocks the low-pH-dependent externalisation of VP1u, which is necessary for endosomal escape and nuclear targeting. However, in the case of B19V, VP1u is already externalised upon receptor binding. Destabilisation of endosomal membranes by CQ facilitates the escape of mature B19V into the cytosol and its transport to the cell nucleus where the virus replicates.

Despite the increased resistance of *Plasmodium falciparum* to CQ, the drug continues to be used in many endemic areas. Our data suggest that a non-4-aminoquinoline drug should be preferred to partner the artemisinin-based combination therapy, which is the current World Health Organization-recommended treatment for uncomplicated malaria.
Uncoating parvovirus’ true nature

Detailed research from scientists at the University of Bern and CSL Behring in Switzerland is uncovering the mechanisms of inactivation and infection of parvoviruses, which have a wide health and medical impact.

**HUMAN PLASMA CONTAINS** myriad proteins which possess properties that can be used to manufacture various lifesaving medical therapies. However, plasma may contain pathogens such as viruses which can cause disease in humans. One such virus is human parvovirus B19 (B19V).

First discovered in 1975 in a study published in The Lancet, B19V is typically associated with erythema infectiosum or fifth disease. The virus is transmitted primarily via the respiratory system and is common; serologic evidence shows past infection in 40-60 per cent of young adults and more than 80 per cent of elderly people. In the main, the infection passes with no serious repercussions, but this is not the case in all instances: individuals with pre-existing blood or immune system disorders, as well as pregnant women, are likely to experience more serious complications.

**A MODEL VIRUS**

Parvoviruses, B19V in particular, are the current interest of the research group of Professor Christoph Kempf and Dr Carlos Ros from the Department of Chemistry and Biochemistry at the University of Bern, Switzerland. They are especially successful as models for unknown and emergent viruses. The research group is working to develop optimal methods to eliminate viruses from human plasma without disturbing valuable plasma proteins.

Before such methods can be developed and implemented, it was vital to gain greater insight into the mechanisms by which parvoviruses infect a cell and reach the blood. Two recent studies published by the research group in the Journal of Virology have addressed mechanisms of parvovirus cell entry, intracellular trafficking and uncoating. The latter refers to the process by which the protein coat of the virus is removed, releasing the viral genome and making the viral genes available for transcription. Inside the cell, the robust parvovirus capsid becomes uncoated under conditions that do not disturb the surrounding cellular proteins. Ros explains: “We want to identify these mechanisms on the molecular level in order to propose improved strategies to further reduce the residual risk of transmission of pathogens to the patient”.

**EXPOSING THE INACCESSIBLE**

The group’s most recent study, published last autumn, explored the role of the VP1 unique region (VP1u) in B19V uptake. It was demonstrated that this immunodominant part of the viral capsid, at first inaccessible, is exposed once the capsid becomes attached to the globoside receptor. Ros describes the group’s method: “We expressed this region as a recombinant protein. Purified recombinant VP1u binds and internalises cells. By means of truncations and specific antibodies, we identified the amino acid sequence of VP1u mediating the internalisation process”.

The importance of this work lies in the fact that it identified VP1u, specifically the N-terminal region, as the viral protein responsible for the internalisation of B19V. Solid evidence was obtained which demonstrated that, upon interaction with the primary receptor globoside, VP1u undergoes a structural change that exposes it to the cell surface.

**Immunofluorescence image showing a parvovirus-infected cell. Viruses (green) accumulate inside lysosomes (red) appearing in yellow.**
rearrangement. This in turn allows VP1u to interact with a host membrane component, finally facilitating B19V internalisation.

Unlike other paroviruses, B19V internalisation is severely restricted, because the expression of the VP1u interacting receptor seems limited to erythroid progenitor cells in the bone marrow. Currently, the group is working to identify the cell receptor that allows the internalisation of B19V. Continuing with this line of enquiry is essential, as it provides the basis for understanding the extremely narrow viral tropism and pathogenesis necessary for the development of antiviral treatments that have the capacity to intervene in the early stages of B19V infection.

EARLY STAGES OF INFECTION

A previous study, published in 2012, sought to characterise these early steps of B19V infection. It demonstrated that B19V interacts with its receptor globoside on lipid rafts, which are specialised plasma membrane microdomains. The study showed that B19V internalisation occur via calthrin-coated pits, and that acidic endosomal pH is required for endosomal escape.

Earlier still, an investigation published in 2007 looked at the molecular mechanism underlying B19V inactivation in comparison with other paroviruses. In general, paroviruses are highly resistant to inactivation treatments, but B19V is more sensitive. After application of wet heat and low pH, it was found that these treatments instigate inactivation in B19V by prompting the release of the viral DNA, leaving the capsid intact but empty and therefore not infectious. This process occurs to a much lesser extend in other paroviruses, rendering them far more resistant to similar inactivation treatments.

THE CHLOROQUINE TEST

The research group has found an unexpected effect of chloroquine (CQ) in B19V infection. Apart from its antimalarial effects, CQ is a potent antiviral. It is successful in the alkalinisation of the endosomal vesicles and therefore very effective against paroviruses, which depend on endosomal acidification for the structural rearrangements required for cell entry.

However, as the research team has demonstrated over the past few years, B19V is unique in that it is not dependent on low endosomal pH for the conformational change. It is also more sensitive to acid damage than other viruses. Consequently, CQ-associated alkalinisation of endosomal vesicles in fact minimises the acidic disintegration of incoming B19V particles. Since CQ destabilises endosome/lysosome membranes, it also facilitates the endosomal escape of B19V, which increases the number of particles that can target nuclei for replication.

FIGHTING ANAEMIA IN THE DEVELOPING WORLD

In collaboration with scientists from Australia and Papua New Guinea (PNG), the team published research in 2010 which monitored children in PNG hospitalised for malaria. Findings confirmed that there is a danger of CQ aggravating B19V-associated anaemia, which is linked with malaria.

The human impact of this unfortunate kinship between B19V and CQ reverberates throughout regions where malaria is endemic.

Although Ros is keen to stress that CQ has its benefits – it is inexpensive and safe as an antimalarial – he believes drug administration policies need to be revised: “In many malaria-endemic regions, CQ is given to febrile children without confirmed malaria,” he divulges. “Paradoxically, fever can actually be due to B19V infection, which is very common. The long terminal elimination half-life of CQ means that conditions favourable to B19V replication may persist for several weeks after dosing.”

More comprehensive clinical studies are needed, but data suggest that a non-4-aminoquinoline drug would minimise the contribution of B19V to severe anaemia. In countries such as PNG, where malaria is endemic and up to 90 per cent of six year olds were found to be B19V seropositive, these developments should be considered seriously.