PERSPECTIVES: BIOMEDICINE

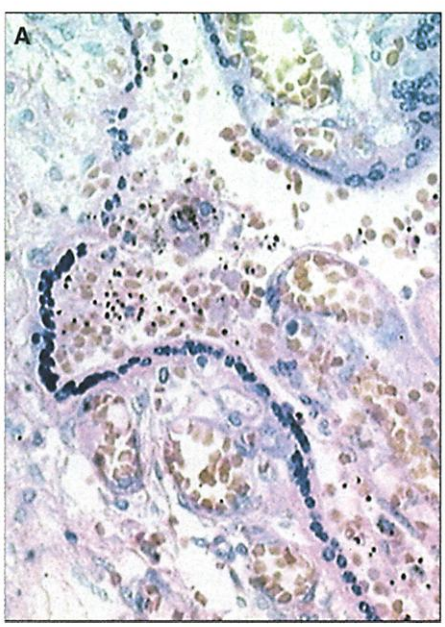
Turncoat Antibodies

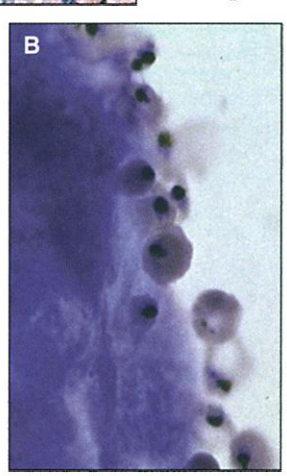
Patrick E. Duffy and Michal Fried

Plasmodium falciparum, the protozoan parasite that causes the most severe form of malaria, is deadly because it causes the red cells that it infects to bind to endothelium and to become sequestered deep within the vascular beds of tissues. Infected red cells also become sequestered in the placenta, where the intervillous (maternal) spaces can be packed with malaria-infected erythrocytes and with phagocytic immune cells called macrophages (see the figure). Earlier work suggested that malaria-infected red cells bind to chondroitin sulfate A (CSA) or hyaluronic acid (HA) expressed by the syncytiotrophoblast (a trophoblastic syncytium formed by the coalescence of trophoblast cells) that lines the placenta. On page 2098 of this issue, Flick *et al.* reveal an additional way in which infected red cells can become sequestered in the placenta. They show that nonimmune immunoglobulin G (IgG) adsorbed on the surface of infected red cells anchors them to Fc receptors expressed by the syncytiotrophoblast (*1*).

Malaria is more severe in women who are pregnant, and parasite adhesion offers a rational explanation for the particularly high susceptibility of women to malarial infection during first pregnancy. By presenting receptors such as CSA that are unavailable elsewhere in the vasculature, the placenta selects parasite-infected red cells with distinct adhesive and antigenic properties. Women may have little exposure to these distinct parasites until their first pregnancy, making primigravidae particularly susceptible. Women develop resistance as they acquire antibodies against CSA-binding parasites, suggesting that a vaccine targeting placental parasites, delivered before first pregnancy, could protect women against malaria once they become pregnant.

A malaria vaccine for pregnant women would be likely to include erythrocyte membrane protein 1 (PfEMP1)—a large, highly variant parasite antigen encoded by the *var* gene family that is expressed on the surface of infected red cells. Each PfEMP1 molecule comprises multiple domains, including Duffy binding–like (DBL) domains and cysteine-rich interdomain regions (CIDRs). Some forms of DBL (*2*, *3*) and CIDR (*3*, *4*) bind to CSA in vitro. In the new work, Flick *et al.* show that another form of DBL can adsorb nonimmune IgG to form a bridge that enables binding of the infected red cell to Fc receptors on the syncytiotrophoblast. Variation in PfEMP1 sequences makes development of a vaccine based on this molecule a daunting task. However, women develop immunity to "pregnancy malaria" relatively quickly (over one to two pregnancies). Furthermore, antibodies that inhibit parasite binding to CSA develop naturally, are associated with resistance to infection, and cross-react with African and Asian parasite isolates (*5*). Thus, the antigens of placental parasites that are targeted by protective immune responses may have conserved epitopes or a limited number of variant forms, making them particularly suitable for use in a vaccine.





Parasite attractions. **(A)** In naturally infected placentas, malaria-infected red blood cells (identified by the dark brown pigment of the parasite) can fill the intervillous spaces of the placenta, and attract inflammatory cells, including macrophages. These pathological findings are associated with severe anemia in the malaria-infected mother and low birth weight in their babies. The intervillous spaces of the placenta are bordered by the syncytiotrophoblast, identified by its dark blue, densely packed nuclei. **(B)** In ex vivo binding assays where parasites are allowed to bind to sections of uninfected placentas, parasitized red blood cells collected from placentas adhere only along the surface of the syncytiotrophoblast, and not elsewhere in the intervillous space, nor on villous stroma.

The most important question raised by Flick *et al.* is whether a pregnancy malaria vaccine will need to target several parasite binding phenotypes in order to protect women. The answer can only come from field studies. In western Kenya where malaria transmission is intense, ex vivo binding assays—in which parasitized red cells are allowed to bind to sections of uninfected placenta—indicate that CSA is the principal or only placental receptor (*6*); studies at other sites also identify CSA as a placental receptor (*7*, *8*). Future field work should assess the separate contributions of CSA, HA, or IgG receptors to sequestration of parasitized red cells in the placenta, and should determine whether the immune response targeting the various binding phenotypes correlates with naturally occurring protection.

Models of in vivo binding have limitations. First, placental parasites are sequestered throughout the intervillous spaces of the placenta in vivo, but they bind only to the syncytiotrophoblast in ex vivo assays (see the figure). This may reflect loss of intervillous material during tissue preparation. Therefore, understanding parasite interactions within the intervillous spaces of the placenta requires alternative approaches. Second, adhesion receptors such as CD36 that are not on the syncytiotrophoblast surface can be expressed within placental stroma and cause artifactual binding in ex vivo assays. Third, CSA or HA preparations are often complex mixtures. For CSA preparations, the degree of sulfation is critical: Highly sulfated forms can fail to support adhesion, whereas low-sulfated forms are optimal for binding (*9*, *10*) and appear on the syncytiotrophoblast and in intervillous spaces (*10*). In HA preparations, CSA is often present as a contaminant, and should be avoided by using pure HA preparations. Fourth, Flick *et al.* show that infected red cells in the placenta have antibodies on their surface, but it is not

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clear whether these are acquired antibodies that recognize infected red cells (*11*) or nonimmune IgG.

The new work raises several questions for future research. Protein A inhibits parasite adhesion although it does not bind to IgG3. This should prompt an investigation of isotype specificity in the interaction between DBL domains and IgG. The authors propose that it is the neonatal Fc receptor that is a placental receptor for infected red cells. However, several studies have concluded that the neonatal Fc receptor is not expressed on the syncytiotrophoblast surface (*12*, *13*), but instead is found in vesicles within the syncytiotrophoblast where it binds to IgG taken up by pinocytosis. Either the cellular localization of neonatal Fc receptors will need to be revisited, or consideration will need to be given to other surface molecules with Fc receptor activity. Furthermore, Fc receptors are expressed throughout the placental villus, so why did malaria-infected red cells coated with antibody adhere only to the syncytiotrophoblast surface? Fc receptors for antibody are expressed in other vascular beds—why would infected red cells coated with antibody be selected only by the placenta, and not by other tissues? Malaria may teach us something new about the accessibility or specificity of antibody receptors.

The helminth *Schistosoma mansoni*, a parasite of humans, is thought to adsorb host antibody onto its surface to avoid immunologic recognition (*14*). In a similar way, *P. falciparum* might benefit from IgG coating the red cells that it infects. If so, this would complement its other strategies for immune evasion, including antigenic variation, direct modulation of the host immune response, and, of course, deep vascular sequestration that permits the parasite to avoid clearance by the spleen. *P. falciparum* possibly emerged only recently as a human pathogen (*15*), but it has proven itself remarkably adept at adjusting to and manipulating its host.

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PERSPECTIVES: TRANSCRIPTION

Transcription Factor IID—Not So Basal After All

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From skin and muscle to nerve and blood, our bodies are composed of more than 200 distinct types of differentiated cells. But how does this profusion of different cell types arise from a single fertilized egg? With a few exceptions, all of our cells contain identical genetic information, and thus the features of each cell must be determined by its pattern of gene expression, that is, which genes are turned "on or "off." The process that switches on the appropriate genes in the correct cells at the right time is, therefore, central to cellular differentiation and the development of multicellular organisms.

Gene expression is controlled predominantly by regulating transcription, the process that copies the gene's DNA instructions into messenger RNA (mRNA), which is then translated into protein. The molecular machinery that drives the transcription of genes comprises RNA polymerase II (the actual enzyme that makes mRNA) and a group of basal or general transcription factors (GTFs): TFIIA, B, D, E, F, and H (*1*). This basal transcription machinery assembles on a DNA sequence, termed the core promoter, located at the beginning of a gene. In addition, regulated gene expression requires DNA sequences, termed enhancers, that bind to sequence-specific DNA-binding proteins (activators), which in turn activate transcription (see the figure). Each gene is controlled by a unique array of binding sites for distinct activators that ensure its expression at the right time and place. The communication between the enhancer-bound activators and the basal transcription machinery depends on a third class of transcription factors, the so-called coactivators (*2*). It is generally believed that a universal, invariant basal transcription apparatus integrates the signals from enhancer-bound gene-specific activators expressed only in particular cells. However, with the flurry of recent papers (*3*–*9*), including one by Freiman *et al.* (*3*) on page 2084 of this week's issue, it is time to revise and extend this view. The new studies reveal that tissue-specific components of the basal transcription machinery can also be gene-specific regulators of development.

Surprisingly, these studies implicate tissue-specific TFIID-related factors in orchestrating two of the most extraordinary cell-differentiation programs in metazoan organisms, namely, the development of male and female gametes. During the formation of both eggs (oogenesis) and sperm (spermatogenesis) the precursor cells undergo meiosis, a type of cell division that reduces the double set of chromosomes to a single set. At the same time, there are dramatic changes in cell structure and morphology directed by stringently controlled stage-specific gene expression programs. The oocyte, the largest cell in an animal, is extensively prepared and intricately programmed to develop into a new individual. Sperm, on the other hand, are "stripped-down" motile cells tailored for the delivery of DNA to the egg.

TFIID is the prime sequence-specific DNA-binding GTF and forms the scaffold upon which the rest of the basal machinery assembles as a prelude to transcription (*1*). Consequently, binding of TFIID to the core promoter constitutes a critical rate-determining step and a key point at which activators can control transcription. TFIID comprises the TATA box–binding protein (TBP) and about 10 TBP-associated factors (TAF_{II}s) (*2*, *10*–*12*). TBP and most of the TAF_{II}s are highly conserved from yeast to human and are encoded by essential genes. TBP is important for binding of TFIID to the TATA-box; the DNA-binding TAF_{II}s recognize other core promoter elements, such as the initiator and the downstream promoter element (*13*). Selected TAF_{II}s are coactivators and are believed to promote transcription by acting as adaptors, linking activators to the basal machinery (*2*). Moreover, $TAF_{II}250$ harbors three distinct enzymatic activities that are all involved in transcriptional regulation (*2*). To complicate matters further, a subset of TAF_{II}s are also constituents of histone acetyltransferase (HAT) complexes that lack TBP (*11*, *12*).

The first hint of tissue-specific TFIID components was the discovery of $TAF_{II}105$, a subunit of TFIID, in a differentiated human B cell line (*14*). Cloning of

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