OPINION

Antibody-based therapies for malaria

Richard J. Pleass and Anthony A. Holder

Abstract | Antibodies are multifunctional glycoproteins that are found in blood and tissue fluids, and can protect against malaria by binding and neutralizing malaria parasites and preparing them for destruction by immune cells. Important technical advances mean that it is now possible to synthesize antibodies against important *Plasmodium* antigens that could be used for therapeutic purposes. These reagents could be designed to act like a drug and kill parasites directly, or could be used in vaccine strategies to protect individuals from infection. In this article, we discuss the possible therapeutic uses of antibodies in the treatment and prevention of malaria.

Despite control efforts, malaria continues to kill ~2–3 million people each year, 75% of whom are children, and the latest estimates suggest that malaria still kills more people than HIV[1]. The important human malaria parasite *Plasmodium falciparum* has developed resistance to cheap and previously reliable drugs such as chloroquine, and few new drugs are in development. Although a vaccine against malaria is the ultimate goal, its development has been beset with problems, and the search for alternative therapies has now begun to assume greater importance.

The recent publication of the *P. falciparum* genome sequence has given researchers the ability to begin to analyse which molecules are important targets of protective immunity[2]. Immunoglobulins (Igs) have an important role in immunity to malaria, and the passive administration of human Igs from hyperimmune sera can be effective at controlling the disease[3,4]. However, the difficulty in finding immune donors and the risks associated with the use of human blood products have necessitated the introduction of recombinant techniques for the production of antibodies against important parasite molecules for use in prototypic therapeutic and vaccine studies.

As the direction of malaria research begins to shift towards questions of protein structure, function and interaction — in particular at the host–parasite interface — antibodies can also provide important insights into the structure and function of key antigens, for example by identifying epitopes that are best suited for incorporation into vaccines[5]. Importantly, because there are no good animal models for *P. falciparum* malaria, antibodies can offer an inexhaustible supply of reproducible standards for use in neutralization assays to provide *in vitro* correlates of protection for the development of vaccines. Until now, work on human malaria parasites has often used antibodies purified from immune sera in these neutralization tests. However, sera contain a mixture of antibodies, some with inappropriate specificities such as BLOCKING ANTIBODIES[5,6], or can contain ineffective antibody classes with the potential to trigger ineffectual killing responses. These drawbacks can be averted with recombinant engineered human MONOCLONAL ANTIBODIES (mAbs), which are homogeneous with respect to class and target epitope.

Antibodies and effector functions

There are five classes of human Igs, divided into nine subclasses (IgM, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgE and IgD) and all produced by B cells. Each antibody is composed of two pairs of identical heavy (μ, γ1, γ2, γ3, γ4, α1, α2, ε or δ) and light (κ or λ) chains, both containing VARIABLE DOMAINS and CONSTANT DOMAINS (FIG. 1). The variable domains contain three hypervariable or COMPLEMENTARITY-DETERMINING REGIONS, which interact with antigen. The heavy and light chains are held together by covalent and non-covalent bonds, forming a molecule with bilateral symmetry (FIG. 1). The two functional FAB DOMAINS have identical antigen specificity. The primary function of an antibody is to bind antigen, which in malaria can have a direct effect, for example by neutralizing malaria toxin or by preventing the parasite from invading erythrocytes. However, binding to antigen can also be ineffective unless secondary effector functions are activated. This is achieved through the FC REGION, which recruits effector function by binding FC RECEPTORS (FcRs), and/or complement.

There are FcRs for all classes of human antibodies. These receptors are widely distributed on cells of the immune system and, by binding to the Fc region, can couple the humoral immune response and cellular immune response[7,8]. FcRs can trigger or inhibit various cellular processes, including phagocytosis, antioxidant production, ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY and the release of cytokines, chemokines and other inflammatory mediators[7]. Most FcRs are structurally related and comprise a unique ligand-binding chain (α-chain), which is often complexed in the transmembrane region with a dimer of the FcR γ-chain, which is common to many FcRs. Effector mechanisms are signalled through immunoreceptor tyrosine-based activation or inhibitory motifs present in the cytoplasmic regions of either the α-chain or γ-chain.

In addition, it is now known that antibodies can be effective against microorganisms without mediating a direct effect[9]. For example, antibodies can be effective in controlling malaria by reducing the damage that results from excessive host inflammatory responses[10]. Recent work

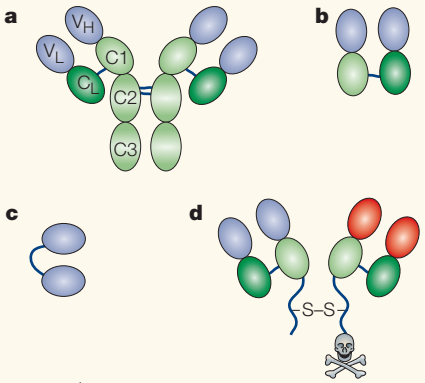

Figure 1 | **Antibody formats.** Schematic representation of an intact antibody (**a**); a Fab fragment (**b**); a single chain variable format (scFv) molecule (**c**), which comprises two variable regions and which is the smallest antigen-recognition unit that can be produced in eukaryotic or prokaryotic expression systems; and a bi-specific $F(ab')_2$ heterodimer (**d**). Both the scFv and $F(ab')_2$ formats can be used to generate recombinant therapeutic molecules. C, constant; H, heavy; L, light; V, variable.

showing that AUTOANTIBODIES AND IMMUNE COMPLEXES (common in malaria) drive B-cell responses through the pattern-recognition receptor (PRR) Toll-like receptor 9 (TLR9) supports this concept for malaria, in which immune-complex deposition in tissues causes pathology[11]. Understanding the biology of the effector mechanisms recruited by antibodies, including FcRs, complement and PRRs, will be an important adjunct to developing optimal antibody-based therapies for malaria[8,12].

Antibody format. Genetic engineering and mammalian expression systems allow the production of human mAbs of a defined class and specificity, to target protective or neutralizing epitopes on the parasite. However, passive administration of mAbs might not always be appropriate, for several important reasons. Whole antibody can potentially bind more than one type of FcR and drive inappropriate immune responses, for example by triggering inhibitory FcRs[8]. A mAb also has to compete with circulating antibodies for FcR binding. This could be important in malaria, as infection induces the production of abundant low-affinity polyclonal IgG and HYPERGAMMAGLOBULINAEMIA[13]. To circumvent these problems, researchers have been assessing the potential of antibody-based fragments produced by manipulating the variable regions of antibody genes (FIG. 1). Single-chain variable fragments (scFvs) that can be formed from two variable regions represent the smallest antigen-recognition unit that can be produced in eukaryotic or prokaryotic expression systems. By linking parasite-specific scFvs to scFvs that recruit human immune effectors, many potential therapeutic molecules can be generated (FIG. 1). In this article, we will explore the various antibody formats and effector functions that might be useful in controlling the malaria parasite.

Antibodies and immunity to malaria

Acquired immunity to malaria occurs only after many years of recurring infection and, although protective against severe clinical disease, it fails to provide sterile immunity. Consequently, in regions where malaria is endemic and transmission intensities are high, young children bear the brunt of the disease. One of the reasons that acquired immunity to malaria is slow to manifest is that clinical immunity results only after continued exposure from multiple infections with many parasites over time. As a result, high-affinity protective antibodies recognizing invariant epitopes of otherwise polymorphic antigens (arising as a result of ANTIGENIC VARIATION) are slow to develop.

However, the importance of antibodies in protection against malaria has been clearly shown. The most convincing evidence for their role in humans comes from clinical studies in which hyper-immune serum or purified Igs from adults substantially modified the course of infection, reducing peak parasitaemias and leading to spontaneous resolution when administered to sick children[3,4]. Maternally derived antibodies can protect neonates and infants from *P. falciparum* and *Plasmodium vivax* infection[14]. In animal models, passive immunization with mAbs[15–17], ADOPTIVE TRANSFER of immunity with B cells[18] and increased susceptibility of B-cell-deficient hosts have all highlighted the crucial role of antibodies[19]. In clinically immune individuals, the naturally acquired antibodies are mainly from the IgG1 and IgG3 subclasses[20]; however, there is little correlation between the levels of these antibodies and resistance to infection or disease. This is believed to be due in part to antigenic variation and the time taken by individuals to develop acquired immunity. This gradual development of acquired immunity indicates that proteins that undergo antigenic variation (for example, erythrocyte membrane protein 1 (EMP1)) could be vitally important[21]. The role of antibodies in protective immunity is not completely understood, but various mechanisms, including inhibition of merozoite invasion of erythrocytes[16], antibody-mediated phagocytosis through FcR and complement[22] and ANTIBODY-DEPENDENT CELLULAR INHIBITION, are possible[4,20].

Designing therapeutic antibodies

Given the evidence for a role of IgG1 and IgG3 in immunity to malaria, it would seem logical to design therapeutic antibodies belonging to these subclasses. However, both IgG1 and IgG3 can bind FcγRs on cells that do not directly kill *P. falciparum*, including platelets, B cells, endothelial cells and even placental tissue[7,23,24], resulting in the triggering of inhibitory FcγRIIb. This might benefit the parasite, analogous to the effect on tumour cell growth, in which passively administered anti-tumour antibodies enhanced tumour cell growth through FcγRIIb[24]. Another drawback of using IgG for malaria therapy is the high level of parasite-induced IgG in hypergammaglobulinaemia[13]. These antibodies might compete with the passively administered IgG for FcR occupancy, and therefore phagocyte recruitment. The presence of this pre-existing IgG could explain why large doses of antibody have been required to neutralize parasites both *in vitro* and *in vivo*[25].

Other Ig classes should also be considered in the design of therapeutic antibodies. Elevated levels of parasite-specific IgE in asymptomatic individuals are associated with a reduced risk of subsequent clinical malaria[26]. However, IgE also has a role in malaria pathogenesis, and therefore might not be appropriate for use in immunotherapy[27]. Although non-specific binding of IgM is implicated in pathology, such as ROSETTE FORMATION by infected erythrocytes[28], parasite-specific IgM is a potent adjuvant in murine malaria vaccination studies[29]. Immune complexes containing IgM serve as innate microbial recognition receptors, and have recently been shown to be powerful adjuvants, stimulating the development of acquired T-cell-mediated immunity, probably through their interaction with Fcα/μR[30]. Therefore, recombinant parasite-specific IgM might have potential in IgM-adjuvant-based malaria vaccines.

We believe that targeting the FcαR (CD89) with IgA could offer the greatest potential for controlling malaria with therapeutic antibodies[8]. Unlike IgM, IgG and IgE, which are all implicated in the pathology associated with rosette formation[28], placental malaria[23] and severe malaria[27], IgA has not been implicated in malaria pathology, arguing for its consideration in antibody therapy. Although a direct role for IgA in killing malaria parasites has not been fully investigated, *Plasmodium*-specific IgA has been detected at high levels in serum[31,32] and breast milk[32,33] in humans from endemic areas. Ligation of the myeloid FcαR induces

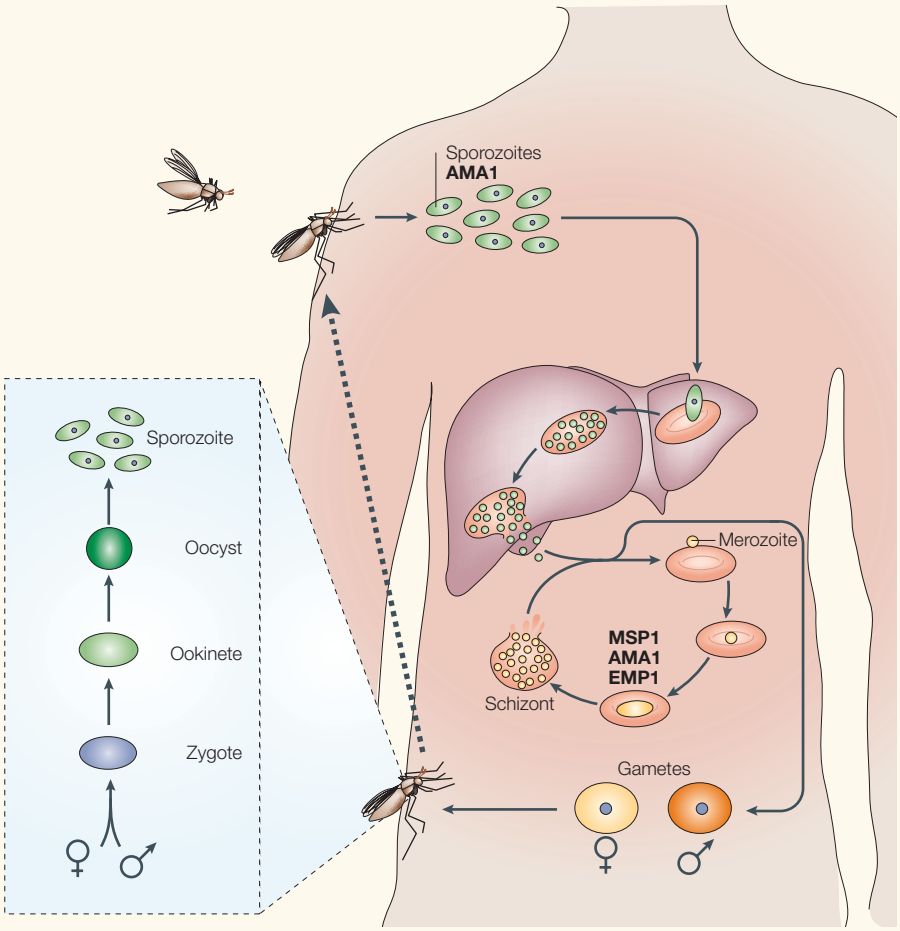

Figure 2 | **The life cycle of *Plasmodium falciparum*.** The life cycle of *Plasmodium falciparum*, showing the parasite stages and antigens that are amenable to antibody-based targeting. Of particular interest are merozoite surface protein 1 (MSP1), apical membrane antigen 1 (AMA1) and erythrocyte membrane protein 1 (EMP1).

cytokine release and can stimulate a respiratory burst[22,34], and FcαR is better than the FcγRs at triggering lysis of antibody-targeted tumours as well as phagocytosis of pathogens coated with antibodies, both in humans and mice[22,35]. Human FcαR is readily available for targeting with therapeutic IgA, as it is constitutively expressed on monocytes, neutrophils and eosinophils as well as KUPFFER CELLS and dendritic cells, making it a good target for antigen presentation[34]. The recent finding that FcαR is a discrete modulator of the immune system that can mediate both anti- and proinflammatory functions indicates that further exploration of the role of IgA in malaria could be important[36].

Potential target molecules

The *Plasmodium* parasite has a complex life cycle (FIG. 2). The problem for researchers interested in using antibodies, either for therapeutic or vaccination purposes, is which life-cycle stage, and which antigens from the bewildering assortment available, to target. Although this question has not been fully addressed, the obvious target for therapeutic antibodies is the asexual blood stage (ABS), which is responsible for the pathology of malaria. Parasites in the ABS express many surface and secreted proteins that have been proposed as good vaccine candidates[37]. Three of these ABS antigens are considered below, together with the likely mechanisms of action of specific therapeutic antibodies. Although we focus solely on the ABS, other parasite stages might also express suitable antigens for targeting by recombinant antibodies.

Merozoite surface protein 1 (MSP1). MSP1 is an attractive target for antibody therapy. Animals actively or passively immunized against MSP1 can be protected against parasite challenge, and mAbs to MSP1 can block parasite invasion of erythrocytes *in vitro*[5,6,15,38]. MSP1 undergoes dual proteolytic processing. It is initially cleaved (primary processing) into multiple fragments that form a protein complex on the merozoite surface. Then, at erythrocyte invasion, the protein is cleaved again (secondary processing) and shed from the surface, except for a C-terminal 19-kDa polypeptide ($MSP1_{19}$) that comprises two epidermal growth factor (EGF)-like domains and which is carried into the newly invaded erythrocyte. Importantly, although MSP1 is polymorphic, sequence variation in $MSP1_{19}$ between *P. falciparum* isolates is limited[39], possibly because the structure is constrained by its function[40]. Naturally exposed individuals have antibodies specific for $MSP1_{19}$ that correlate with resistance to clinical malaria[41] and are the major erythrocyte-invasion-inhibitory factor *in vitro*[42]. Novel genetic approaches, including LINKAGE-GROUP SELECTION, have also identified MSP1 as an important target of immunity[43]. The fine specificity of the antibodies binding to $MSP1_{19}$ is important for their function, and only some $MSP1_{19}$-specific mAbs inhibit invasion, which they do by inhibiting the secondary processing of MSP1 (REF. 44). The conformational epitopes recognized by such antibodies have been defined both broadly and in detail[5,45–47]. Such antibodies, in addition to causing direct elimination of the parasite, should also present conserved $MSP1_{19}$ epitopes to antigen-presenting cells, stimulating cellular immunity and limiting parasite proliferation.

To explore further the potential of $MSP1_{19}$-targeted antibodies, recombinant human chimaeric IgG1 and IgA1 against $MSP1_{19}$ from the rodent malaria parasite *Plasmodium yoelii*[22] have been generated (FIG. 1). These reagents, in particular the IgA1, triggered efficient *in vitro* phagocytosis of merozoites by human neutrophils. However, in contrast to the parental mouse IgG2b (from which both the chimaeric antibodies were derived), both recombinant IgG1 and IgA1 failed to protect against parasite challenge *in vivo* when used to passively immunize mice, despite possessing comparable affinity for $MSP1_{19}$ (REF. 22). This failure of the engineered antibodies to protect *in vivo* probably stems from an inability of their human Fc regions to trigger murine effector mechanisms, and indicates that blocking $MSP1_{19}$ function by steric hindrance alone is insufficient to provide protection. These reagents will permit evaluation of human effector mechanisms *in vivo*, by using mice transgenic for human FcR and complement genes. Such *in vivo* experiments are not possible with human malaria parasites, including *P. falciparum*, which are adapted for life in their human hosts.

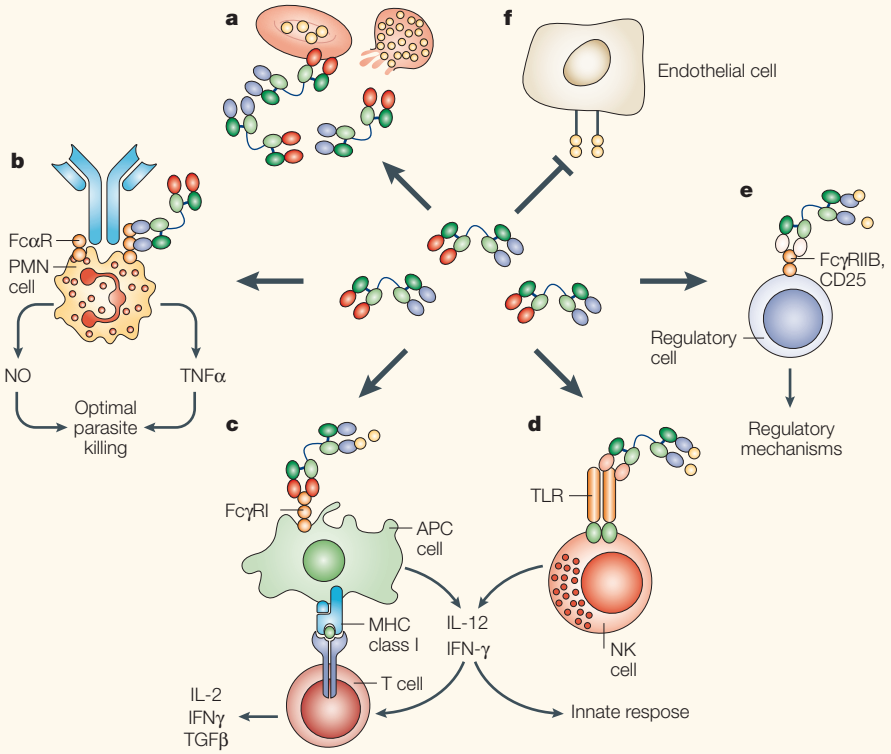

Figure 3 | **Bispecific antibodies (BsAbs).** BsAbs can be designed in various formats to control the malaria parasite. Injected BsAbs bind to key antigens on the merozoite or infected erythrocyte surface. This can inhibit progression of the disease by inhibiting proteolytic cleavage of antigens required for erythrocyte entry by the parasite (**a**). BsAbs for optimal killing and phagocytosis of merozoites that bind the Fcα receptor (FcαR) (this is the optimal FcR in terms of bioavailability and killing power) expressed on monocytes, macrophages, polymorphonuclear leukocytes (PMNs), NK (natural killer) cells and some dendritic cells (DCs) are designed to bind the FcR outside the receptor's natural binding site for antibody, to circumvent interference by serum immunoglobulin (**b**). BsAb can deliver malaria antigen to professional antigen-presenting cells (APCs) including DCs (**c**) or to NK cells through Toll-like receptors (TLRs) to optimally harness innate immune responses (**d**). Regulatory cells can also be specifically targeted, thereby modifying the course of disease (**e**). High-affinity binding BsAb can be designed to prevent pathology (**f**), for example by blocking the interaction of erythrocyte membrane protein 1 (EMP1) with endothelial receptors such as CD36 or intercellular adhesion molecule-1 (ICAM1). IFN-γ, interferon- γ; IL, interleukin; NO, nitric oxide; TGF, transforming growth factor-β; TNFα, tumour-necrosis factor- α; T_{reg}, regulatory T cell.

Apical membrane antigen 1 (AMA1). AMA1 is a promising vaccine candidate that has induced protection in both rodent and non-human primate models of malaria[48]. Antibodies to AMA1 have also been shown to inhibit erythrocyte invasion and parasite growth *in vitro*[49], indicating that AMA1 is also an attractive target for therapeutic antibodies. As AMA1 is expressed by infectious sporozoites from the mosquito as well as merozoites, targeting AMA1 has the additional advantage of attacking two stages of the parasite's life cycle[50] (FIG. 2).

Erythrocyte membrane protein 1 (EMP1). EMP1 is a highly polymorphic, parasite-derived antigen that is expressed on the surface of infected erythrocytes and undergoes antigenic variation[21]. EMP1 is also responsible for cytoadhesion of infected erythrocytes to vascular endothelial cells, leading to parasite sequestration in the brain, kidneys and placenta, with particular importance in cerebral and pregnancy-associated malaria. High-affinity therapeutic antibodies that bind to functionally important regions of EMP1 could be developed to block cytoadhesion and facilitate phagocytosis of infected red blood cells by antibody binding to FcR on the surface of macrophages. Antibodies from patients' serum have been shown to bind conserved linear epitopes in EMP1 (REF. 51), suggesting that the development of therapeutic antibodies to target infected erythrocytes is a real possibility.

Novel antibody-based formats

There are many potential advantages to using 'designer antibodies' over conventional mAbs for therapeutic applications. The antibody format available is limited only by the imagination of the investigator, and the choice will depend on the desired outcome (FIG. 1). Reagents can be designed that are optimal for parasite killing, whereas others might be better at presenting antigen for the development of immunity in the context of a vaccine (FIG. 3). Ideally, antibody-based therapies should be designed with both outcomes in mind. For malariologists, particular approaches might be most relevant (FIG. 3). For example, to circumvent the competition from pre-existing antibodies for FcR occupancy, bispecific antibody-based reagents (FIGS 1,3) can be created that bind to the parasite antigen of choice and to FcRs outside their natural antibody-binding site, allowing the recruitment of immune cells already saturated in antibody[22] (FIG. 3). This application might be particularly important in the case of malaria, because of the hypergammaglobulinaemia associated with this disease[13]. This specific targeting also means that smaller quantities of antibody can be given in treatment, therefore providing additional savings in terms of cost.

Bispecific antibodies (BsAbs) have been developed to redirect or enhance immune effector activity towards particular targets, including tumour cells and pathogens, and they are clinically promising[52,53]. The prototypic BsAb consists of two covalently linked $F(ab')_2$ fragments, with one fragment targeting the pathogen or tumour cell and the other fragment targeting a trigger molecule (FIG. 1). We are investigating the BsAb format. Using recombinant methods, we have coupled the variable domains from a mAb recognizing *P. yoelii* $MSP1_{19}$ to the variable domains from a mAb recognizing human FcγR1, through a flexible peptide linker. This BsAb binds FcγR1 outside the high-affinity IgG-binding site, and simultaneous engagement of $MSP1_{19}$ and FcγRI by this molecule induced a potent neutrophil respiratory burst[22]. These promising results obtained with FcγRI BsAb in the *P. yoelii* model system have provided an impetus to generate BsAbs against *P. falciparum* that target other FcRs.

Other uses of BsAbs can be envisaged. For example, given the importance of innate immune responses in driving adaptive immunity, it might be of interest to link *P. falciparum* $MSP1_{19}$ to TLRs (FIG. 3). The ability of BsAbs to bind two different epitopes simultaneously indicates that a BsAb could be designed to inhibit the proteolytic cleavage of the MSP1 complex that is necessary for erythrocyte invasion by binding to either side of the cleavage site (FIG. 3). A bispecific reagent for malaria therapy has recently been

developed for the treatment of *P. falciparum* malaria[54]. This BsAb targeted the CD3 antigen of human T cells and $MSP1_{19}$. In cooperation with T cells, the BsAb induced a significant increase in merozoite phagocytosis and inhibition of *P. falciparum* growth *in vitro*. Trispecific antibodies that bind $MSP1_{19}$, AMA1 and FcR could be useful, as this type of reagent is effective against tumours and might offer enhanced protection against malaria[55].

The antibody immunotherapy for malaria (AIM) programme is generating intact chimaeric and fully human antibodies against key malaria antigens with which to compare the effectiveness of the BsAbs. These are generated by cloning variable domains from hybridomas or from antibody repertoire phage display libraries (generated from immune Gambian adults) and linking them to human constant domains in suitable expression vectors. Although there is clear evidence that chimaeric antibodies are less immunogenic than murine mAbs, there is little evidence to support claims for further humanization to avoid the antiglobulin response, perceived as an important problem in the clinical development of therapeutic antibodies[56].

Although passive antibody therapy can confer immediate protection against malaria, rapid and efficient methods for generating the high-affinity neutralizing mAbs required for use in these novel formats have been slower in arriving. However, considerable progress has now been made in this area, including improved immortalization of human B cells by Epstein–Barr virus[57], versatile high-throughput phage, prokaryotic and fungal expression systems for selecting high-affinity human antibody-binding fragments[58–60], and immunization of transgenic mice carrying human Ig loci, coupled with hybridoma technology[61].

Another exciting development has been the discovery of alternative antigen-binding scaffolds to the paired heavy and light chains from mammalian antibody repertoires (FIG. 1). For example, both camelids (camels, llamas and related species) and sharks produce single variable domains capable of high-affinity binding to buried targets[62,63]. This finding could have a major impact for antibody therapy in malaria, as some target molecules with cavities or clefts might be refractory to conventional antibody development. The camelid repertoire has been used to generate antibodies that can target enzyme active sites and similar targets[64,65], indicating that such an approach could be used to inhibit key malaria enzymes, for example those involved in MSP1 and AMA1 processing.

Glossary

ADOPTIVE TRANSFER
The transfer of protective immunity with cells taken from an immunized donor and injected into a naive recipient.

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY
A mechanism by which natural killer cells are targeted to IgG-coated cells, resulting in the lysis of the antibody-coated cells.

ANTIBODY-DEPENDENT CELLULAR INHIBITION
The ability of antibodies, in cooperation with monocytes, to limit parasite growth during *in vitro* culture. The exact mechanisms have yet to be identified.

ANTIGENIC VARIATION
The ability of parasites to minimize or avoid immune attack by the rapid turnover or regular change in the antigens exposed on their surfaces.

AUTOANTIBODIES AND IMMUNE COMPLEXES
Autoantibodies are generated by polyclonal activation of B cells on infection. They cause pathology by crossreacting with host tissues such as erythrocytes and DNA, and are common in malaria. Immune complexes are the product of an antigen–antibody reaction, which can also contain components of the complement system. They can be deposited in tissues, where they cause disease.

BLOCKING ANTIBODIES
Block the binding and function of inhibitory monoclonal antibodies.

COMPLEMENTARITY-DETERMINING REGION
The most variable regions of antibodies, which form loops that make contact with specific antigens. There are three such regions (CDR1, CDR2 and CDR3) in each variable domain.

CONSTANT DOMAINS
The relatively invariant parts of immunoglobulin heavy and light chains.

FAB DOMAIN
The part of an antibody molecule that contains the antigen-binding site, consisting of a light chain and part of the heavy chain; it is produced by enzymatic digestion.

FC REGION
The region of an antibody that is responsible for binding to antibody receptors on cells and the C1q component of complement.

FC RECEPTOR
Surface molecules on various cells that bind to the Fc regions of immunoglobulins, thereby initiating effector functions.

HYPERGAMMAGLOBULINAEMIA
In addition to the rise in specific antibodies, many parasite infections, including malaria, provoke the production of high titres of non-specific antibody, so-called hypergammaglobulinaemia. Much of this increase is probably due to antigens released from the malaria parasite acting as polyclonal mitogens (substances that cause cells, particularly lymphocytes, to undergo cell division) for B cells.

KUPFFER CELLS
Macrophages present in the liver. Kupffer cells are derived from blood monocytes, and phagocytose particles that enter the liver sinusoids.

LINKAGE-GROUP SELECTION
A genetic method for identifying genes underlying any selectable phenotype in malaria parasites, including strain-specific protective immunity.

MONOCLONAL ANTIBODY
An antibody that is directed against a specific epitope and is produced by a single clone of B cells or a single hybridoma cell line, which is formed by the fusion of a lymphocyte cell with a myeloma cell.

ROSETTE FORMATION
In malaria, infected red blood cells sometimes bind to uninfected red blood cells to form clumps, called rosettes. The rosettes can obstruct flow in small blood vessels and lead to tissue damage and severe malaria disease. IgM contributes to rosette formation by stabilizing the interaction between infected and uninfected erythrocytes in a manner that is not completely understood.

VARIABLE DOMAINS
The N-terminal domains of antibody heavy and light chains, which become recombined with appropriate sets of D and J genes during B-cell development.

The selection and affinity maturation of single-antigen-receptor variable domains from sharks targeting *P. falciparum* AMA1 has been described and developed for diagnosis[66].

Antibodies can also be manipulated for use in innovative vaccine applications, for example targeting liver-stage parasites, where antibody is believed to have a minor role in immunity. 'Troybodies' have been developed that incorporate several T-cell epitopes into antibodies with specificity for the PRR CD14. This approach involves exchanging loops in Ig constant domains with single copies of well-defined T-cell epitopes. Troybodies were found to be 100–100,000 times more efficient than synthetic peptide or native protein at inducing T-cell responses[67]. These reagents might therefore be usefully incorporated into malaria vaccination strategies that currently use various systems such as viral vectors for epitope delivery.

Antibodies can also be used as vehicles for the targeted delivery of antimalarial drugs. The development of drug resistance through the widespread consumption of antimalarials and adverse drug reactions arising from their toxicity are important global problems. Antibodies can deliver drugs directly to the parasite, thereby optimizing their pharmacokinetics and reducing the risk of adverse drug reactions. A plethora of molecules have been attached to antibodies, including drugs, enzymes, toxins, viruses and radionuclides. Although these approaches have been shown to be effective in cancer therapy, they remain untested in malaria.

Concluding remarks

Antibodies are believed to represent ~25% of all proteins in clinical trials[68]. This interest in antibodies as therapeutic entities stems not only from a detailed understanding of their structure and immunological function but also from technological advances that have allowed their manipulation and expression. As a result, at least 18 antibodies have approval for use from the US Food and Drug Administration, and at least 100 are in clinical trials worldwide, the majority for the treatment of cancer, transplant rejection or autoimmune disease[69]. Despite these successes, none is in development for any tropical disease, including malaria. Most pharmaceutical companies shy away from investing in diseases they believe will not provide a healthy dividend for the shareholder. The seriousness of the health situation demands that the primary priority should be to find novel effective therapies. Antibody-based reagents and therapies are believed to be expensive and therefore inappropriate for a disease mainly affecting poor and marginalized populations. However, the pace of technological developments, especially in protein expression (for example in plants), should bring costs down substantially. In addition, these novel approaches could be useful as prophylaxis for the increasing number of non-immune individuals travelling to affected countries. Although it is unlikely that antibody-based prophylaxis will be made available to the millions of people at risk from malaria, antibody-based therapies could usefully be incorporated into the hospital setting, for patients non-responsive to conventional drug regimens. Importantly, antibody-based therapies have 'proof of concept' in clinical settings, ensuring that the development time from laboratory to patient would be significantly reduced and the costs constrained, a luxury unavailable to other prospective therapies. Moreover, antibodies are natural adjuvants that can present important epitopes to antigen-presenting cells, providing a 'vaccine effect', and thereby limiting proliferation of the parasite on re-infection. This potential one-off treatment could be cost effective compared to conventional vaccination strategies, which often require complex multiple boosting regimens and harsh adjuvants. The parasite is becoming resistant to conventional antimalarials, and therefore the urgency of the situation leaves little room for complacency.

Richard J. Pleass is at the Institute of Genetics, School of Biology, University of Nottingham, Nottingham, NG7 2UH, UK.

Anthony A. Holder is at the Division of Parasitology, National Institute for Medical Research, Mill Hill, London, NW7 1AA, UK.

Correspondence to R.J.P.
e-mail: Richard.Pleass@nottingham.ac.uk

doi:10.1038/nrmicro1267

1. Snow, R. W. *et al.* The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* **434**, 214–217 (2005).
2. Gardner, M. J. *et al.* Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **419**, 498–511 (2002).
3. Cohen, S. *et al*. γ-globulin and acquired immunity to human malaria. *Nature* **192**, 733–737 (1961).
4. Bouharoun-Tayoun, H. *et al*. Antibodies that protect humans against *Plasmodium falciparum* blood stages do not on their own inhibit parasite growth and invasion *in vitro*, but act in cooperation with monocytes. *J. Exp. Med*. **172**, 1633–1641 (1990).
5. Uthaipibull, C. *et al*. Inhibitory and blocking monoclonal antibody epitopes on merozoite surface protein 1 of the malaria parasite *Plasmodium falciparum*. *J. Mol. Biol*. **307**, 1381–1394 (2001).
6. Guevara Patino, J. A. *et al.* Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human Abs. *J. Exp. Med.* **186**, 1689–1699 (1997).
7. Hulett, M. D. & Hogarth, P. M. Molecular basis of Fc receptor function. *Adv. Immunol.* **57**, 1–127 (1994).
8. Shi, J., McIntosh, R. & Pleass, R. J. Antibody and Fc-receptor based therapeutics for malaria. *Clin. Sci.* (in the press).
9. Casadevall, A. & Pirofski, L. A. Antibody-mediated regulation of cellular immunity and the inflammatory response. *Trends Immunol.* **24**, 474–478 (2003).
10. Samuelsson, A., Towers, T. L. & Ravetch, J. V. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* **291**, 484–486 (2001).
11. Leadbetter, E. A. *et al*. Chromatin–IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* **416**, 603–607 (2002).
12. McGuinness, D. H., Dehal, P. K. & Pleass, R. J. Pattern recognition molecules and innate immunity to parasites. *Trends Parasitol*. **19**, 312–319 (2003).
13. Abele, D. C. *et al*. Alterations in serum proteins and 19S antibody production during the course of induced malaria infections in man. *Am. J. Trop. Med. Hyg*. **14**, 191–197 (1965).
14. Logie, D. E. *et al.* Plasma immunoglobulin concentrations in mothers and newborn children with special reference to placental malaria: studies in the Gambia, Nigeria, and Switzerland. *Bull. World Health Organ.* **49**, 547–554 (1973).
15. Siddiqui, W. A. *et al.* Merozoite surface coat precursor protein completely protects Aotus monkeys against *Plasmodium falciparum* malaria. *Proc. Natl Acad. Sci. USA* **84**, 3014–3018 (1987).
16. Quinn, T. C. & Wyler, D. J. Mechanisms of action of hyperimmune serum in mediating protective immunity to rodent malaria (*Plasmodium berghei*). *J. Immunol.* **123**, 2245–2249 (1979).
17. Badell, E. *et al*. Human malaria in immunocompromised mice: an *in vivo* model to study defense mechanisms against *Plasmodium falciparum*. *J. Exp. Med*. **192**, 1653–1660 (2000).
18. Langhorne, J. *et al*. A role for B cells in the development of T cell helper function in a malaria infection in mice. *Proc. Natl Acad. Sci. USA* **95**, 1730–1734 (1998).
19. Von der Weid, T. *et al*. Gene-targeted mice lacking B cells are unable to eliminate a blood stage malaria infection. *J. Immunol.* **156**, 2510–2516 (1996).
20. Bouharoun-Tayoun, H., Oeuvray, C., Lunel, F. & Druilhe, P. Mechanisms underlying the monocyte-mediated antibody-dependent killing of *Plasmodium falciparum* asexual blood stages. *J. Exp. Med.* **182**, 409–418 (1995).
21. Bull, P. C. *et al*. Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Nature Med*. **4**, 358–360 (1998).
22. Pleass, R. J. *et al*. Novel antimalarial antibodies highlight the importance of the antibody Fc region in mediating protection. *Blood* **102**, 4424–4430 (2003).
23. Flick, K. C. *et al*. Role of nonimmune IgG bound to PfEMP1 in placental malaria. *Science* **293**, 2098–2100 (2001).
24. Clynes, R. A. *et al.* Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. *Nature Med.* **6**, 443–446 (2000).
25. Saul, A. & Miller, L. H. A robust neutralization test for *Plasmodium falciparum* malaria. *J. Exp. Med.* **193**, 51–54 (2001).
26. Bereczky, S. *et al.* Elevated anti-malarial IgE in asymptomatic individuals is associated with reduced risk for subsequent clinical malaria. *Int. J. Parasitol*. **34**, 935–942 (2004).
27. Perlmann, P. *et al*. Immunoglobulin E, a pathogenic factor in *Plasmodium falciparum* malaria. *Infect. Immun*. **65**, 116–121 (1997).
28. Creasey, A. M. *et al*. Nonspecific immunoglobulin M binding and chondroitin sulfate-A binding are linked phenotypes of *Plasmodium falciparum* isolates implicated in malaria during pregnancy. *Infect. Immun.* **71**, 4767–4771 (2003).
29. Harte, P. G., Cooke, A. & Playfair, J. H. L. *et al*. Specific monoclonal IgM is a potent adjuvant in murine malaria vaccination. *Nature* **302**, 256–258 (1983).
30. Stäger, S. *et al*. Natural antibodies and complement are endogenous adjuvants for vaccine-induced $CD8^+$ T-cell responses. *Nature Med.* **9**, 1287–1292 (2003).
31. Biswas, S., Saxena, Q. B., Roy, A. & Kubilan, L. Naturally occuring *Plasmodium* specific IgA antibodies in humans from a malaria endemic area. *J. Biosci.* **20**, 453–460 (1995).
32. Kassim, O. O. *et al*. Inhibitory factors in breastmilk, maternal and infant sera against *in vitro* growth of *Plasmodium falciparum* malaria parasite. *J. Trop. Pediatr.* **46**, 92–96 (2000).
33. Leke, R. G. *et al*. Identification of anti-*Plasmodium falciparum* antibodies in human breast milk. *Scand. J. Immunol. Suppl.* **11**, 17–22 (1992).
34. Monteiro, R. C. & van de Winkel, J. G. IgA Fc receptors. *Annu. Rev. Immunol.* **21**, 177–204 (2003).
35. Otten, M. A. *et al*. Immature neutrophils mediate tumor cell killing via IgA but not IgG Fc receptors. *J. Immunol*. **174**, 5472–5480 (2005).
36. Pasquier, B. *et al.* Identification of FcαRI as an inhibitory receptor that controls inflammation: dual role of FcRγ ITAM. *Immunity* **22**, 31–42 (2005).
37. Good, M. F., Kaslow, D. C. & Miller, L. H. Pathways and strategies for developing a malaria blood-stage vaccine. *Annu. Rev. Immunol.* **16**, 57–87 (1998).
38. Blackman, M. J. *et al*. A single fragment of a malaria merozoite surface protein remains on the parasite during red cell invasion and is the target of invasion inhibiting antibodies. *J. Exp. Med.* **172**, 379–382 (1990).
39. Holder, A. A. *et al.* A malaria merozoite surface protein (MSP1)-structure, processing and function. *Mem. Inst. Oswaldo Cruz* **87**, 37–42 (1992).
40. O'Donnell, R. A. *et al.* Functional conservation of the malaria vaccine AgMSP-1_{19} across distantly related *Plasmodium* species. *Nature Med.* **6**, 91–95 (2000).
41. Egan, A. F. *et al*. Clinical immunity to *Plasmodium falciparum* malaria is associated with serum antibodies to the 19-kDa C-terminal fragment of the merozoite surface antigen, PfMSP-1. *J. Infect. Dis.* **173**, 765–769 (1996).
42. O'Donnell, R. A. *et al.* Antibodies against merozoite surface protein (MSP)-1_{19} are a major component of the invasion-inhibitory response in individuals immune to malaria. *J. Exp. Med.* **193**, 1403–1412 (2001).
43. Martinelli, A. *et al.* A genetic approach to the *de novo* identification of targets of strain-specific immunity in malaria parasites. *Proc. Natl Acad. Sci. USA* **102**, 814–819 (2005).
44. Blackman, M. J. *et al*. Antibodies inhibit the protease mediated processing of a malaria merozoite surface protein. *J. Exp. Med*. **180**, 389–393 (1994).
45. Dekker, C. *et al*. Inhibitory and neutral antibodies to *Plasmodium falciparum* $MSP1_{19}$ form ring structures with their antigen. *Mol. Biochem. Parasitol*. **137**, 143–149 (2004).
46. Morgan, W. D. *et al*. Precise epitope mapping of malaria parasite inhibitory antibodies by TROSY NMR cross-saturation. *Biochemistry* **44**, 518–523 (2005).
47. Morgan, W. D. *et al*. Malaria parasite-inhibitory antibody epitopes on *Plasmodium falciparum* merozoite surface protein 1 (19) mapped by TROSYS NMR. *Mol. Biochem. Parasitol*. **138**, 29–36 (2004).
48. Stowers, A. W. *et al*. Vaccination of monkeys with recombinant *Plasmodium falciparum* apical membrane Ag1 confers protection against blood-stage malaria. *Infect. Immun*. **70**, 6961–6967 (2002).
49. Kocken, C. H. *et al.* High-level expression of the malaria blood-stage vaccine candidate *Plasmodium falciparum* apical membrane Ag1 and induction of antibodies that inhibit erythrocyte invasion. *Infect. Immun.* **70**, 4471–4476 (2002).

50. Silvie, O. *et al*. A role for apical membrane Ag1 during invasion of hepatocytes by *Plasmodium falciparum* sporozoites. *J. Biol. Chem.* **279**, 9490–9496 (2004).
51. Staalso, T. *et al*. Antibody reactivity to conserved linear epitopes of *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1*). Immunol. Lett.* **60**, 121–126 (1998).
52. Fanger, M. *Bispecific Antibodies* (Springer–Verlag, New York, 1995).
53. Segal, D. M. *et al*. Special issue: BsAbs. *J. Immunol. Methods* **248**, 1–194 (2001).
54. Yoshida, S. *et al*. T-cell activation and cytokine production via a bispecific single-chain antibody fragment targeted to blood-stage malaria parasites. *Blood* **101**, 2300–2306 (2003).
55. Ruf, P. & Lindhofer, H. Induction of a long-lasting antitumor immunity by a trifunctional bispecific antibody. *Blood* **98**, 2526–2534 (2001).
56. Clark, M. Antibody humanization: a case of 'Emperor's new clothes'? *Trends Immunol*. **21**, 397–402 (2000).
57. Traggiai, E. *et al*. An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. *Nature Med*. **10**, 871–875 (2004).
58. Bradbury, A. R. M. & Marks, J. D. Antibodies from phage antibody libraries. *J. Immunol. Methods* **290**, 29–49 (2004).
59. Fernández, L. A. Prokaryotic expression of antibodies and affibodies. *Curr. Opin. Biotech*. **15**, 364–373 (2004).
60. Feldhaus, M. J. & Siegel, R. W. Yeast display of antibody fragments: a discovery and characterization platform. *J. Immunol. Methods* **290**, 69–80 (2004).
61. Green, L. L. Antibody engineering via genetic engineering of the mouse: xenomouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies. *J. Immunol. Methods*. **231**, 11–23 (1999).
62. Nguyen, V. K., Desmyter, A. & Muyldermans, S. Functional heavy-chain antibodies in *Camelidae. Adv. Immunol.* **79**, 261–296 (2001).
63. Stanfield, R. L. *et al*. Crystal structure of a shark single-domain antibody V region in complex with lysozyme. *Science* **305**, 1770–1773 (2004).
64. Parren, P. W. & Burton, D. R. The antiviral activity of antibodies *in vitro* and *in vivo*. *Adv. Immunol.* **77**, 195–262 (2001).
65. Peretz, D. *et al*. Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. *Nature* **412**, 739–743 (2001).
66. Nuttall, S. D. *et al*. Selection and affinity maturation of IgNAR variable domains targeting *Plasmodium falciparum* AMA1. *Proteins* **55**, 187–197 (2004).
67. Tunheim, G. *et al*. Human CD14 is an efficient target for recombinant immunoglobulin vaccine constructs that deliver T cell epitopes. *J. Leukoc. Biol*. **77**, 303–310 (2005).
68. Hudson, P. J. & Souriau, C. Engineered antibodies. *Nature Med.* **9**, 129–134 (2003).
69. Hollinger, P. & Hudson, P. J. Engineered antibody fragments and the rise of single domains. *Nature Biotechnol.* **23**, 1126–1136 (2005).

Acknowledgements
We thank the Medical Research Council, The European Union, The Sir Halley Stewart Trust and The Wellcome Trust for support.

Competing interests statement
The authors declare no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
Entrez: http://www.ncbi.nlm.nih.gov/Entrez
Epstein–Barr virus | *Plasmodium falciparum* | *Plasmodium vivax* | *Plasmodium yoelii*
Swiss-Prot: http://www.expasy.ch
AMA1 | CD14 | CD89 | EMP1 | MSP1 | TLR9
CDC Infectious Disease Information:
http://www.cdc.gov/ncidod/diseases/index.htm
HIV | malaria

FURTHER INFORMATION
Richard J. Pleass's homepage: http://www.nottingham.ac.uk/biology/contact/academics/pleass/overview.phtml
Anthony A. Holder's homepage:
http://www.nimr.mrc.ac.uk/parasitol
Access to this interactive links box is free online.

OPINION — COMMONHEALTH OF NATIONS

Do we have the architecture for health aid right? Increasing global aid effectiveness

Tore Godal

Abstract | The advent of the new millennium has witnessed the embracing of a different perspective on global health aid. New and innovative mechanisms in health-aid financing are leading to new opportunities, focused on greater innovation, risk taking and speed. However, these opportunities might not fully materialize if the traditional approaches of channeling and using funds are followed. To maximize global aid effectiveness and to have a realistic chance of achieving the Millennium Development Goals, the implementation of a holistic approach to the global architecture of health aid will be essential.

Throughout the 1990s, research has increasingly shown that investments in health are key to poverty reduction[1,2]. Moreover, there was an increasing global understanding of the interdependency of health and wealth and the need to achieve optimal levels of both. In 2000, Gro Harlem Brundtland, then Director-General of the World Health Organization (WHO), encapsulated the new appreciation when she stated that "there was a period in development thinking when spending on public services, such as health and education, would have to wait. Good health was a luxury, only to be achieved when countries had developed a particular level of physical infrastructure and established a certain economic strength … Experience and research over the past few years have shown that such thinking was at best simplistic and at worst plainly wrong … Good health is not only an important concern for individuals, it plays a central role in achieving sustainable economic growth and effective use of resources"[3].

Reflecting this revised thinking, new mechanisms to facilitate improved global health financing were created. These represented new ways of channeling and using funds for health. Notable among the first of these new initiatives was the Global Alliance for Vaccines and Immunization (GAVI) with its financing arm, the Vaccine Fund (VF). This was followed by the bigger Global Fund to fight AIDS, Tuberculosis and Malaria. These instruments, created through worldwide collaborative efforts, identified new approaches and mobilized new money. In the process, they established a clear mission to deliver the most cost-effective health commodities to where they were most needed. Their underlying characteristics were based on certain assumptions relating to the broader field of health aid. Now, a few years later, it is appropriate to examine the establishment of these instruments and understand the broader architecture in health aid as it prevails today. Health-related humanitarian action has not been included in this analysis.

Health aid is composed of an extensive and heterogeneous set of contributing components and stakeholders. Nevertheless, there is a broad-based agreement on what is required. First, there is a need to focus on the poorest countries and the poorest sections of the community within those countries, and second, there is a requirement to reach these populations with the most cost-effective and easy-to-use interventions. To achieve sustainable results, there is also a general consensus that the process of aid allocation needs to become more long-term in outlook, more predictable, more effective (through harmonization and alignment) and more accountable (through objective monitoring and evaluation mechanisms). However, with regard to the approaches that are required to achieve these goals, there are strong differences in opinion. Moreover, despite the mobilization of relatively large amounts of new funds for health aid over the past few years, the level of resources available to meet basic, global public-health needs falls woefully short of requirements. In 2001, the Macroeconomic and Health Commission estimated that about US$30 billion were needed in aid per year ($27 billion by 2007, rising to $38 billion annually by 2015)[4]. But current health-related annual transfers, despite the new funds