Thalassemia: the long road from the bedside through the laboratory to the community

David Weatherall

I owe my long interest in inherited blood diseases and tropical medicine to a series of characteristically bizarre decisions by the British Army. In 1958, 2 years after qualifying in medicine from Liverpool University, I was drafted for 2 years of compulsory National Service. Terrified of flying, snakes and bullets, I volunteered to serve in the UK. So it was that a few weeks later, and after a long and eventful voyage on a troop ship that ran aground in the Suez Canal, I found myself in charge of the Children's Ward in the British Military Hospital in Singapore. At that time, the hospital served the soldiers and families of the Commonwealth forces who were engaged in the long war against the Chinese communists in Malaya, as it was then called. One of my first patients was a Nepalese child whose father was serving in a Gurkha regiment. She had been profoundly anemic from the first year of life and had been kept alive by monthly blood transfusions. After a long struggle, and with the help of Frank Vella, a biochemist from Singapore University, we found that this child had thalassemia, an inherited blood disease that was thought at the time to be restricted to Mediterranean populations.

I spent the second year of my National Service working in a military hospital in Taiping, close to the Thai border where the remaining communist terrorists were being rounded up. There I saw a wide spectrum of tropical diseases, and, in my spare time and with a primitive electrophoresis setup comprising car batteries and filter paper, searched for more individuals with thalassemia.

David Weatherall is at the Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford, UK.
e-mail: liz.rose@imm.ox.ac.uk

After my discharge from the army, I went to the US to work at Johns Hopkins University, spending time in the medical genetics, hematology and biophysics departments. By 1960 it was known that human hemoglobin (Hb) consists of four pairs of peptide chains—two α-chains and two β-chains ($\alpha_2\beta_2$); in fetal life, the main hemoglobin—HbF—has two α-chains and two γ-chains ($\alpha_2\gamma_2$). It was also known that sickle cell anemia results from an amino acid substitution in the β-globin chain, and there were also hints that the cause of thalassemia might involve the α- or β-globin-chain genes. My first work in Baltimore, carried out in Ned Boyer's laboratory, was to trudge around the hospitals collecting cord blood to look for hemoglobin variants that would clarify the genetic regulation of α-globin synthesis in fetal and adult life and that, incidentally, led to the finding of a mild form of thalassemia in many African-American infants. But it was clear that further progress in understanding the cause of thalassemia would require the development of methods for studying hemoglobin synthesis *in vitro*.

Because of the paucity of red blood cell precursors capable of synthesizing hemoglobin in the blood of normal people or people with thalassemia, it took a while to define the conditions in which it was possible to obtain linear incorporation of radioactive amino acids into hemoglobin for periods of 90 minutes or more. When this approach was applied to blood samples from children with thalassemia, it appeared as though there was unequal labeling of the α- and β-globin chains. The problem was that there was no method for separating them with anything close to a quantitative yield.

A lucky break was to come, however. Two English protein chemists, John Clegg and Michael Naughton, graduates of Fred Sanger's laboratory in Cambridge, UK, were working in the biophysics department at Johns Hopkins at that time. For reasons that are still obscure to me, they took on the unenviable task of trying to educate me into the basics of protein chemistry. Clegg wondered whether, by adapting methods he had used for separating other protein chains in his doctoral thesis work, we could use a similar approach to separate globin chains. It was remarkably successful, and we were able to examine

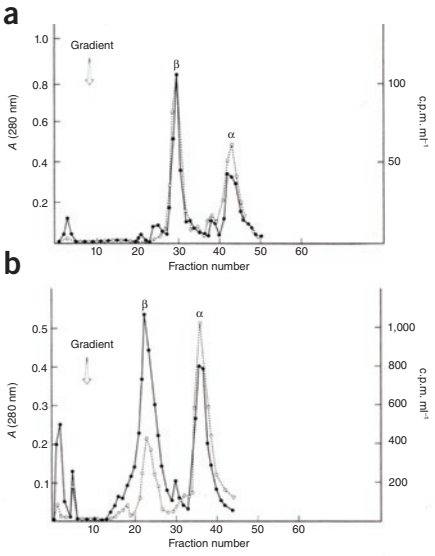

Figure 1 Globin synthesis in thalassemia. (**a**) Labeled globin from a nonthalassemic individual. (**b**) Labeled globin from an intact red blood cell lysate from an individual with β-thalassemia. The continuous black line shows the α- and β-chains, and the broken line represents the incorporation of radioactivity. It later became possible to separate the γ-chains of HbF from the β-chains. Reproduced from ref. 1.

normal and thalassemic labeled globins and show that the basic defect in thalassemia is unbalanced globin-chain synthesis, with excess α-chains produced in β-thalassemia and excess β-chains in α-thalassemia[1] (**Fig. 1**). This method was subsequently used by many groups to define the different forms of thalassemia and their pathology. And, just a few years later, it was used to diagnose thalassemia during fetal life as an approach to prenatal diagnosis, an advance that led to a marked reduction in the births of babies with severe thalassemia in several countries over the succeeding years.

After returning to England in the mid-1960s and writing the first edition of *The Thalassaemia Syndromes*, which has reappeared in several editions over the years[2] as a guide to workers in the field, we established a hemoglobin research group, first in Liverpool and later in Oxford. In the early days, and in collaboration with Robert Williamson and John Paul, we were able to show deletions as the cause of severe forms of α-, β- and δβ-thalassemia. Also, after studying a family with an unusual form of α-thalassemia referred to us by Paul Milner from Jamaica, we were able to characterize thalassemia due to mutations in the α-globin chain termination codon[3]. Later on, these and unrelated mutations were found to be carried by up to 4% of the population of Thailand and to be the cause of α-thalassemia in many other populations.

As direct analysis of DNA became possible, we focused our attention on the remarkable diversity in the structure of the α-globin genes, the numerous mutations that produce the diverse clinical forms of α-thalassemia and the feasibility of early prenatal diagnosis by fetal DNA analysis. We also identified a completely new class of α-thalassemias associated with mental retardation that resulted from subtelomeric deletions affecting the α-globin genes on chromosome 16 or mutations of a gene on the X chromosome. Later on, the first of these two classes led to the recognition of submicroscopic, subtelomeric deletions as a relatively common cause of mental retardation[4].

By the late 1970s, we had developed a team of outstanding scientists supported by the UK Medical Research Council and the Wellcome Trust, including John Clegg, Bill Wood, Douglas Higgs, Swee Lay Thein, Kathryn Robson, John Old, Andrew Wilkie and Richard Gibbons. Later, our interests expanded into the evolutionary biology of the hemoglobin disorders; in particular, to ask why they are so common. In 1949, John B.S. Haldane had suggested that the thalassemias might occur at a high frequency because of heterozygote resistance to severe malaria[5]. Until the molecular era, this was difficult to prove. However, in a series of studies carried out in the Pacific Islands and in Papua New Guinea, our students Adrian Hill and Jonathan Flint, together with Angela and Steve Allen, were able to show without any doubt that the α-thalassemias are highly protective against malaria caused by *Plasmodium falciparum*[6]. Some of our other students, notably Tom and Kathryn Williams, who had taken part in this work, continued to work in this field in East Africa and, aside from replicating these results, described new epistatic interactions between the different hemoglobin variants in Africa with respect to malaria susceptibility.

Throughout these years, we and others continued to explore the reasons for the remarkable clinical diversity of the thalassemias. In the case of the β-thalassemias, for example, it turned out that the co-inheritance of α-thalassemia has an ameliorating effect on the phenotype by reducing the degree of globin chain imbalance, whereas the inheritance of more α-chain genes than usual has the opposite effect. Similarly, an inherited ability to make more γ-chains of fetal hemoglobin results in a milder phenotype. Many of the complications of the disease are also modified by genetic factors; there are differences in the patterns of adaptation to anemia at different ages, and the environment, notably exposure to malaria[7], also modifies the phenotype (**Fig. 2**). Clearly, this so-called 'simple' monogenic disease is anything but simple, an important message for those who are now doing battle with the complexities of the genetic component of common diseases[8].

By the early 1980s, it was clear that the molecular technology that was proving so effective for studying the hemoglobin disorders was going to be applicable to many fields of medical research in the future. As

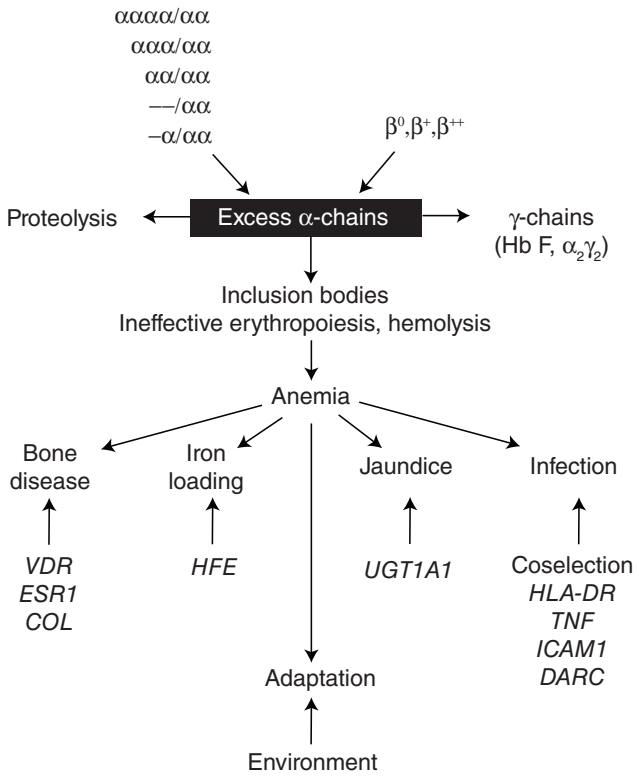

Figure 2 Mechanisms for the phenotypic diversity of β-thalassemia. The basic pathology of the disease results from excess α-chains damaging red blood cell precursors and red blood cells. This excess can be modified by different levels of severity of reduction in β-chain production, variable numbers of α-chains resulting from deletion or expansion of the α-globin gene loci, varying ability to produce HbF after birth and, possibly, different rates of removal of α-chains by proteolysis. The many complications of the resulting anemia can also be modified by genetic variability. Coselection indicates that variants of these genes and many others produce different responses to infection among different populations. *VDR*, vitamin D receptor; *ESR1*, estrogen receptor-1; *COL*, collagen; *HFE*, locus for hereditary hemochromatosis; *UGT1A1*, UDP glucuronyltransferase involved in bilirubin metabolism; *HLA-DR*, major histocompatibility complex locus; *TNF*, tumor necrosis factor; *ICAM1*, intercellular adhesion molecule-1; *DARC*, Duffy antigen receptor for chemokines.

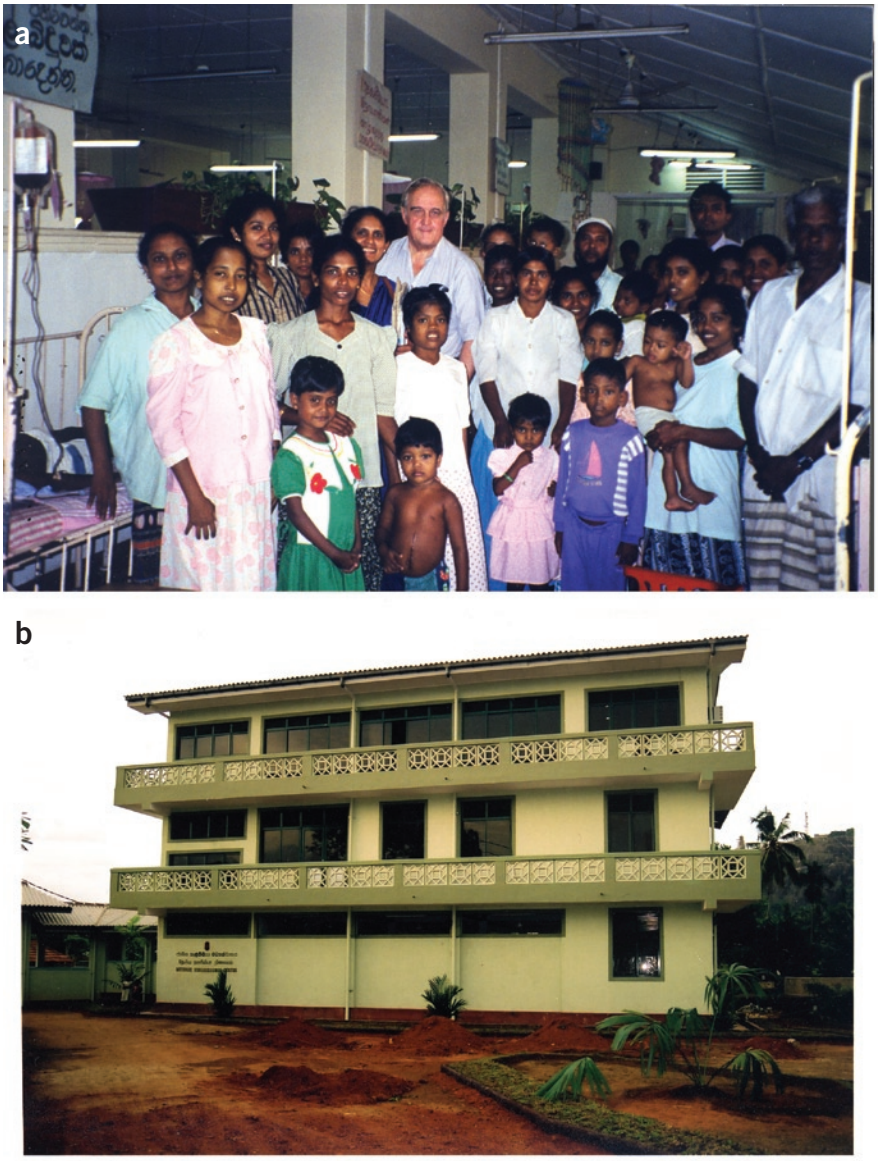

Figure 3 Kurunegala, Sri Lanka. (**a**) The author with a group of staff and thalassemic children in the Children's Ward in the District Hospital at Kurunegala, Sri Lanka. A small alcove behind the children's ward was used to transfuse a population of more than 600 children with thalassemia before the building of the National Thalassaemia Centre. (**b**) The National Thalassaemia Centre, attached to the pediatric ward, offers transfusion facilities for young children on the ground floor and older children on the middle floor. The top floor provides facilities for genetic counseling, basic hematological studies and small group seminars.

there were few places where young clinicians could be trained in this technology, or where young scientists could apply it to clinical problems, we decided to establish an Institute of Molecular Medicine in Oxford, where young people of both backgrounds could mingle, obtain training and bring their particular clinical problems into an environment in which molecular technology was available. The Institute opened in 1989 and now houses over 400 scientists.

As early as the 1960s, it was already apparent that the inherited disorders of hemoglobin occur widely throughout the tropical world. In 1967, the World Health Organization (WHO) asked me to go on an extensive tour of Asia to report back on the occurrence of thalassemia and facilities for its diagnosis and care there. On the basis of this experience, we developed partnerships with several countries, partly for training staff in the clinical and diagnostic aspects of thalassemia, but in many cases for long-term associations to develop collaborative research programs. In international health jargon, these 'North-South' partnerships, some of which have been sustained for many years, have been a cost-effective and productive way of helping countries such as Cyprus, India, Burma (Myanmar), Thailand, Indonesia, South Vietnam, Sri Lanka and Jamaica.

The potential value of sustained partnerships of this type is exemplified by our relationship with Sri Lanka, which has extended over 15 years and during which we have had invaluable support from Nancy Olivieri and her staff in Toronto. As well as developing a long-term research program, it has been possible to carry out a considerable amount of capacity development in Sri Lanka, including building a National Treatment Centre (**Fig. 3**) and an institute for DNA diagnostics in one of the medical schools. It has also resulted in the establishment of a national thalassemia control program.

Currently, the inherited hemoglobin disorders pose an increasingly serious global health problem. Although their high frequency reflects natural selection against malaria, their incidence is augmented by the large numbers of consanguineous marriages in many of the high-frequency countries. Advances in nutrition and public health, which have reduced childhood mortality in developing countries, have also led to an increased prevalence of the inherited hemoglobin disorders; babies with thalassemia or sickle cell anemia who would otherwise have died early in life are now surviving. So, the annual numbers of births of babies with inherited hemoglobin disorders is in excess of 300,000 (refs. 9,10). Recent estimates suggest that, if the life expectancy of African children with sickle-cell anemia doubles, soon there will be some six million children with this condition in sub-Saharan Africa[9].

In 2002, the WHO asked me to write a report on the potential value of genomics for global health in the future[11]. In the recommendations I pointed out the proven value of the North-South partnerships and suggested that the next logical development would be South-South partnerships, that is, links between countries that have developed expertise in the management of these genetic diseases and those where no such skills exist. We are in the process of testing this model in Asia, where we have developed an international network trying to form partnerships with countries such ash Thailand and India, where expertise in the diagnosis and management of these diseases exists, with adjacent countries, including Cambodia and Bangladesh, that do not possess such skills. Although plans for these developments are well advanced, they are extremely difficult to fund. Although both government and charitable funding agencies are willing to accept that there is a substantial

research component in such ventures, they are often unwilling to accept the expenses that are also involved in the essential capacity-building aspects of these programs. This problem is exacerbated by the fact that the WHO, other nongovernmental organizations and the major international charities have, with a few exceptions, shown no interest in genetic disease, focusing almost entirely on the major communicable diseases.

There is some light on the horizon, however. Our Asian Thalassemia Network is well on the way to being established, and there are recent reports of similar interactions for the management of sickle cell anemia in Africa. If the governments of the developed countries can be persuaded that partnerships of this type are a cost-effective way of helping the developing countries, and that some of their aid should therefore be turned in this direction, there is some hope for the future for the control and management of these increasingly common diseases in the poorer countries of the world.

Taking a broader view, at Oxford we have developed similar long-term partnerships for research and capacity building in other aspects of tropical medicine; our partnership with Thailand recently celebrated its thirtieth year. Besides producing international leaders in tropical medicine research, these connections have led to important developments in the management and control of common diseases such as malaria. Although other universities and related organizations have moved in this direction, there is still enormous scope for closer interactions based on these models between universities in the rich countries and those in the developing world. It is crucial that governments, national and international funding bodies and all those who are concerned with improving the level of health globally understand this message.

COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.

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