lished within 0.5 msec of its inception (*13*), must reflect the rearrangement of highly polarizable macromolecules in a fashion that is both submicroscopic and uniform or uniformly periodic along a myofibrillar domain with a mean length of eight sarcomeres in the specimen shown.

This is an unexpected result. Electron microscopy of fixed and stained specimens teaches that the structural unit of striated muscle is not the myofibril, but the sarcomere (*5*). Each sarcomere is invested with longitudinal and transverse SR elements (*14*), and these elements are in communication, at the level of each sarcomere, with the transverse tubular T system, which communicates to the interior of the fiber the depolarization conveyed by the action potential along the sarcolemma (*15*). The invaginations of the sarcolemma giving rise to the T tubules (*16*) have the same longitudinal spacing as the sarcomeres themselves (*16, 17*). On the basis of such structural periodicity, it might be expected that, as the action potential sweeps over the sarcolemma and inward through the T system, the activation that breaks out in its wake would be synchronous or stochastic. If it were stochastic, individual structural units randomly located in the wake would develop activity randomly in time. Successive differential images of the process would have a "salt-and-pepper" appearance until a large fraction of all structural units had become active. In synchronous activation contiguous structural units would become uniformly and simultaneously active over polymyofibrillar regions of length $v_s\Delta t \simeq 400$ μm and breadth $v_r\Delta t \simeq 20$ μm, where v_s (~ 140 cm/sec) and v_r (~ 7 cm/sec) are the axial (17°C) (*18*) and radial (20°C) (*7*) velocities of propagation of the action potential, respectively, and Δt is the time resolution of the observation (~ 0.2 msec in Fig. 3). Neither of these expected outcomes is compatible with our holographic images, which show that the activation that first appears is both segmental and sparse.

Departures from the idealized structures we have sketched are known. Among them are occasional longitudinal elements (*19*) and culs-de-sac in the T system (*20*) and helicoids in both the T system and the sarcomeric registration (*20*). The longitudinal elements and culs-de-sac are very short (*19*) and the helicoids may attain lengths of eight striations longitudinally, but all these irregularities are far less numerous (*20*) than our bright filaments. Thus a proper accounting for the segmentalization appears more likely to be found at the level of the myofibrillar dynamics, or possibly in the distribution of transport in the SR or T systems, than at the level of static structure. A hint that its origin may, with pharmacological assistance, prove identifiable is furnished by our finding that the exposure of a fiber to Ringer solution containing bovine serum albumin not only alters the temporal course of its activation (*12*) but also markedly shortens the filaments that shine in its differential images.

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References and Notes

1. R. L. Powell and K. A. Stetson, *J. Opt. Soc. Am.* **55**, 1593 (1965).
2. L. O. Heflinger, R. F. Wuerker, R. E. Brooks, *J. Appl. Phys.* **37**, 642 (1966).
3. A representative set of applications can be found in G. von Bally, Ed., *Holography in Medicine and Biology* (Springer-Verlag, Berlin, 1979).
4. M. Sharnoff, R. W. Henry, D. M. J. Bellezza, *Biophys. J.* **21**, 109a (1978).
5. An informative collection of definitions, drawings, and photographs is given by D. S. Smith [*Muscle* (Academic Press, New York, 1972)].
6. This situation can be produced by stimulating a fiber locally at one end and observing the other end before its invasion by the action potential. In our experiments this created an interval of several milliseconds during which the myofibrils at the unstimulated end were being passively stretched by the active contraction of those at the stimulated end (*18*).
7. H. Gonzales-Serratos, *J. Physiol. (London)* **212**, 777 (1971).
8. B. A. Mobley and B. R. Eisenberg, *J. Gen. Physiol.* **66**, 31 (1975).
9. J. R. Bendall, *Nature (London)* **170**, 1058 (1952).
10. J. Borejdo and S. Putnam, *Biochim. Biophys. Acta* **459**, 578 (1977).
11. A. F. Huxley and R. Niedergerke, *J. Physiol. (London)* **144**, 403 (1958).
12. L. Hill, *ibid.* **266**, 677 (1977).
13. This is shown in Figs. 1 and 3 and is confirmed by unbalanced images made as in Fig. 2 but later during contraction. The process requires only about 1 percent of the time required for peak tension to develop during a twitch.
14. L. D. Peachey, *J. Cell Biol.* **25**, 209 (1965).
15. J. Bastian and S. Nakajima, *J. Gen. Physiol.* **63**, 257 (1974).
16. C. Franzini-Armstrong, L. Landmesser, G. Pilar, *J. Cell Biol.* **64**, 493 (1974).
17. A. F. Huxley and R. E. Taylor, *J. Physiol. (London)* **144**, 426 (1958).
18. P. Fatt and B. Katz, *ibid.* **115**, 320 (1951).
19. B. Eisenberg, *J. Cell Biol.* **55**, 68a (1972).
20. L. D. Peachey and B. R. Eisenberg, *Biophys. J.* **22**, 145 (1978).
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Functional Expression of a Cloned I-Aβ^k Gene in B-Lymphoma Cells

Abstract. *The immune response genes of the mouse encode two cell-surface glycoproteins, I-A and I-E, that play critical roles in determining the animal's immune responsiveness. The I-A antigen contains two chains, α and β. A cloned β-chain gene, I-Aβ^k, was introduced into B-lymphoma cells that express I-A^d. The transfected gene was successfully expressed on the cell surface of the recipient cells and was functional in stimulating allospecific T cells.*

The I-region of the major histocompatibility complex (MHC) of the mouse encodes the I-A and I-E polymorphic cell-surface glycoproteins referred to as Ia antigens or class II antigens. Each Ia antigen consists of three noncovalently linked polypeptide chains—the α (~ 34 kilodaltons), β (~ 28 kilodaltons), and the invariant chain (~ 30 kilodaltons) (*1*). Ia antigens are expressed primarily on B cells and on antigen-presenting cells such as macrophages and dendritic cells and play a major role in cell-cell interaction and in regulation of the immune response (*2*). To gain a better understanding of the mechanism of Ia antigen regulation, we introduced genes encoding the I-A antigen into mouse B-lymphoma cells. We now report the successful expression of transfected I-Aβ^k (*3*) gene product on the cell surface of antigen-presenting B-lymphoma cells. The expressed I-Aβ^k chain was functional in stimulating allospecific T cells.

Genes of class I antigens of the MHC have been successfully transfected into L cells. Transfected class I genes were expressed on the cell surface of L cells and were able to function as restriction elements in T-cell–specific cytotoxic responses against viruses (*4, 5*). Expression of a functional Ia antigen from transfected class II MHC genes presented a more difficult problem. Although Ia genes can be expressed in L cells (*6, 7*), they are not fully functional. Therefore, we introduced the cloned I-Aβ^k gene into BALB/c B-lymphoma cells M12.4.1 and A20.2J (*8*), which express I-A^d-encoded antigens and are potent Ia-restricted antigen-presenting cells (*9*). These cells should have all the machinery necessary to assemble functional I-A antigens. The successful transfection of M12.4.1 and A20.2J cells with the I-Aβ^k gene should result in a hybrid I-A$^{k/d}$ molecule formed by the pairing of the I-A^d α chain from the B-lymphoma cell with the transfected I-A^k β chain.

The I-Aβ^k gene was introduced into B

cells after calcium phosphate precipitation (*10*) of the cloned DNA and selection for neomycin resistance (Figs. 1 and 2). Four to five weeks after transfection, stable transformants were screened for the expression of the I-Aβ^k gene by radioimmunoassay or by use of the fluorescence-activated cell sorter (FACS). A panel of ten monoclonal antibodies specific for different epitopes of the I-A^k molecule was used for detecting gene expression. Five of these monoclonal antibodies were able to bind to M12.4.1 cells transfected with the I-Aβ^k gene (Fig. 2C) but not to the parent M12.4.1 cells. The transfected M12.βk3 cells are unique in that, unlike F_1 hybrid cells, they express the β chain but not the α chain of I-A^k. Such a cell is valuable in definitively assigning the specificities of the monoclonal antibodies to either the α or the β chain of I-A^k. Thus, monoclonal antibodies 10.2.16, 39B, 39E, 40A, 40B (Fig. 2), and 11-3.25 (data not shown) recognize epitopes on the β chain of I-A^k as suggested earlier (*11, 12*). Similarly, the monoclonal antibodies that do not recognize the transfected M12.βk3 cells must recognize epitopes on the I-A^k α chain or epitopes generated by the pairing of the α^k and β^k chains. Monoclonal antibodies 39C, 39J, 11.5.2, and 26.7.11 are of this type (data not shown).

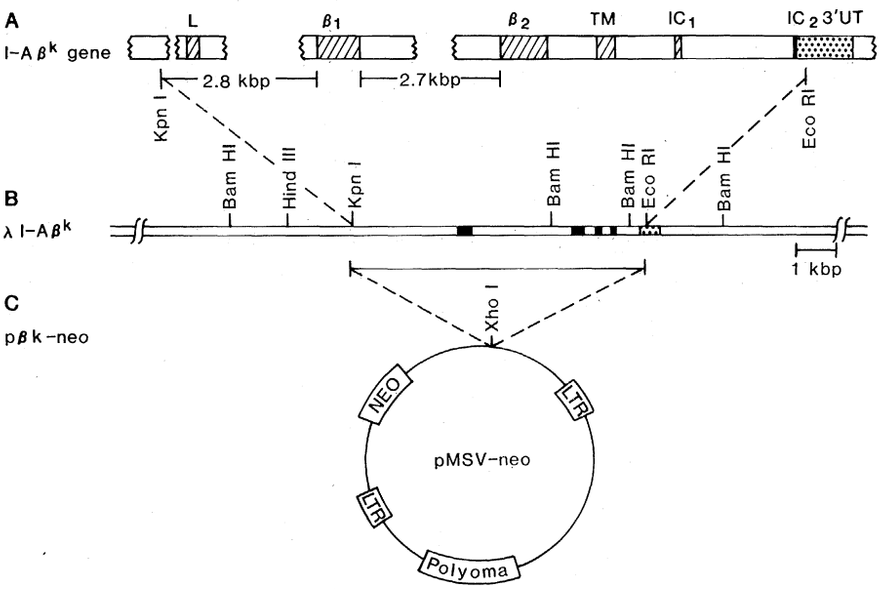

Fig. 1. (A) The organization of I-Aβ^k gene. The exons of I-Aβ^k genes are represented by hatched bars in the coding region and by a stippled bar in the 3′ untranslated (3′UT) region (*15*). The exon encoding the leader is labeled *L*; the exons encoding the two extracellular domains are labeled β_1 and β_2; the transmembrane region is labeled *TM*; and the intracytoplasmic regions are labeled IC_1 and IC_2. The 3′UT region is contiguous with IC_2. The location of the L segment was predicted from its location in the I-Aβ^d and I-Aβ^b genes (*16, 17*). (B) Partial map of the λ I-Aβ^k phage (*15*). The solid bars indicate the coding sequences. (C) Schematic representation of the plasmid vector pMSV-neo, which contains the neomycin-resistance gene (*NEO*) (*18*), and of the construction of pβk-neo plasmid. The pβk-neo plasmid was formed by joining the neomycin-resistance gene and the I-Aβ^k gene in a single molecule; standard technology was used (*13*). The Kpn I–Eco RI DNA fragment contains the coding sequences of I-Aβ^k gene but is truncated at the 3′UT region. A polyadenylation site was provided by using blunt-end ligation to fuse the I-Aβ^k gene into the unique Xho I restriction site located between the two long terminal repeats (*LTR*) present in pMSV-neo.

The introduction of the I-Aβ^k gene into the B cell led to the expression of an I-A^k–like antigen on the cell surface. To demonstrate that this antigen involves a full-size I-Aβ^k chain, we characterized the I-A antigens by immunoprecipitation. Polyacrylamide gel electrophoresis of ^{125}I-labeled surface proteins showed (data not shown) that the transfected I-A^k β chain expressed on A20.βk20 cells precipitated by monoclonal antibody 11-3.25 had mobility identical to that of the I-A^d β chain of A20.2J or A20.βk20 cells precipitated by MK-D6, a monoclonal antibody specific for the I-A^d antigen. These results strongly suggest that a full-size I-A^k β chain was assembled with the I-A^d α chain and expressed on the cell surface of transfected cells. The successful expression of the β chain after transfection with the constructed plasmid pβk-neo (Fig. 1) suggests that the signals necessary for the expression of the I-Aβ^k gene are located on the 7.8-kilobase Kpn I–Eco RI fragment. Further support for this conclusion was derived from the finding that pβk-neo DNA was able to direct the synthesis of the I-Aβ^k chain regardless of the relative orientation of the Kpn I–Eco RI fragment within the plasmid vector pMSV-neo (data not shown).

Transfection with the I-Aβ^k gene was analyzed at the DNA level by Southern blot analysis (*13*). M12.4.1 and A20.2J

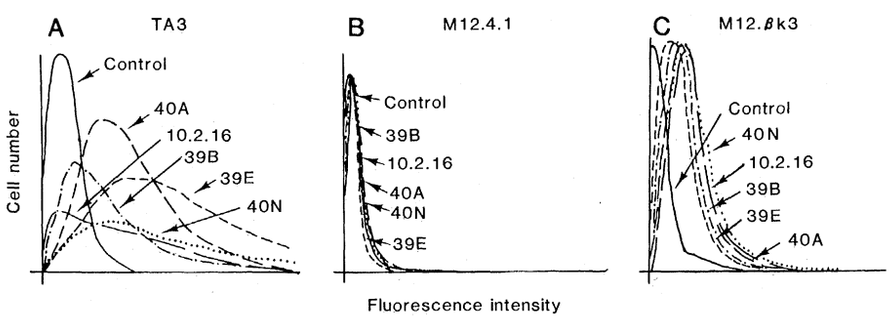

Fig. 2. Fluorescence-activated cell sorter (FACS) analysis of expression of I-Aβ^k chains expressed on the surface of the B-lymphoma cells. M12.4.1 is a cloned BALB/c B-lymphoma cell line expressing I-A^d antigens (*8*). TA3 is a B-cell–B-lymphoma somatic cell hybridoma produced by fusing (BALB/c × A/J)F_1 cells with the M12.4.1 and expresses both I-A^d and I-A^k antigens (*19*). M12.4.1 cells were co-transfected by the calcium phosphate technique (*10*) with λ I-Aβ^k DNA (10 μg) containing the I-Aβ^k gene (Fig. 1) together with pMSV-neo (0.5 μg) DNA containing the neomycin-resistant gene (Fig. 1), which was used as selection marker, and with BALB/c DNA (10 μg) as carrier DNA. Restriction enzyme Sal I was used to linearize pMSV-neo before transfection. The cells were grown in selective media containing G418 (Geneticin, Gibco) (0.8 mg/ml) for 5 to 6 weeks. Neomycin-resistant clones were screened for expression of I-Aβ^k chain by use of 11-3.25, a monoclonal antibody to I-A^k. The M12.βk3 clone was analyzed by FACS with a panel of monoclonal antibodies to I-A^k. Monoclonal antibodies 39C, 39J, 40A, 40N, 39B, and 39E were developed by Pierres (*11*), and monoclonal antibodies 11.5.2, 26.7.11, and 10.2.16 were developed by Oi *et al.* (*20*). Only those monoclonal antibodies that bind to the transfected cells are shown. Cells (1×10^6) were incubated first with monoclonal antibody to Ia and then with a second antibody (Fab fragment of fluorescein-conjugated goat antibody to mouse immunoglobulin) and then analyzed by flow microfluorometry (Spectrum III; Ortho Diagnostic Systems). Control cells were incubated with fluorescein-conjugated goat antibody to mouse immunoglobulin only.

cells that had been transfected either by phage λ I-Aβ^k DNA or the plasmid vector contained at least one copy of the I-Aβ^k gene per cell (Fig. 3). M12.4.1 cells co-transfected with λ I-Aβ^k DNA contained more than one gene copy per cell. However, regardless of the number of I-Aβ^k gene copies integrated in the genome, all transfected cells expressed the β chain of I-A^k at comparable levels—less than the amount of I-Aβ^k expressed on the surface of the TA3 B-cell hybridoma expressing I-A^k and I-A^d antigens (Fig. 2). The amount of I-A^k antigen expressed on transfected M12.βk3, M12.βk4, and A20.βk20 cells as measured by radioimmunoassay was approximately 50 percent of the amount of I-A^k expressed on TA3 B-hybridoma cells (data not shown). Several explanations can be proposed to explain why the transfected cells do not express more than 50 percent of the amount of β-chain I-A^k found on the TA3 cells, but the answer remains uncertain.

Our results show that B cells can be stably transformed with the I-Aβ^k gene and can successfully express the antigen on the cell surface. The next question was whether these β^k-transfected antigen-presenting cell lines could stimulate alloreactive T cells specific for I-A^k or serve as antigen-presenting cells for I-A^k–restricted antigen-specific T cells. A panel of 12 different T-cell hybridomas as well as several interleukin-2–dependent T-cell clones were tested for their activation in the presence of the M12.βk3 or A20.βk20 transfected cells and the relevant antigen. All T cells tested were either specific for foreign antigen in association with I-A^k–encoded restriction elements or were alloreactive or autoreactive for the I-A^k antigen alone.

Rather surprisingly, transfected cells expressing the I-Aβ^k and I-A^d did not stimulate any of the tested T cells. T-cell hybridomas specific for I-A^k or for antigen associated with I-A^k (hen egg lysozyme, ovalbumin, and keyhole limpet hemocyanin) and T-cell clones specific for influenza virus or purified protein derivative (tuberculin) in association with I-A^k fail to respond to the antigen in the presence of M12.βk3 or A20.βk20 cells. However, transfected M12.βk3 and A20.βk20 cells activated an I-A^d–specific autoreactive T-cell hybridoma, indicating that the transfection had not impaired a generalized function of antigen presentation. The most likely explanation for the failure of the transfected cells to activate antigen-specific T cells was that the T-cell panel tested was specific for antigen in association with the homozygous α^k/β^k dimer, but not with the heterozygous α^d/β^k dimer. That such unusual F_1 hybrid restriction elements exist was shown by Kimoto *et al.* (*14*). We therefore established a BALB/c T-cell line that was enriched for reactivity against the I-A$^{k/d}$ hybrid antigen. The transfected cells were then used to stimulate these T cells. Transfected M12.βk3 and A20.βk20 cells stimulated the BALB/c-derived T-cell line to proliferate (Table 1). The level of stimulation by transfected cells was comparable to that obtained by the semiallogeneic TA3 cells or by (BALB/c × A/J) cells. This stimulation was blocked by a monoclonal antibody to I-A^k. Thus, the transfected I-Aβ^k gene expressed as an α^d/β^k dimer on the cell surface of H-2^d–transfected B cells is functional in specific stimulation of T cells.

Table 1. A BALB/c T-cell line rich in cells reactive against a hybrid I-A$^{k/d}$ molecule was prepared by repeated stimulation of BALB/c T cells with irradiated (BALB/c × A/J)F_1 (CAF_1) spleen cells every 10 to 14 days as described (*14*). The ability of the transfected cells to stimulate the BALB/c T-cell line, was tested by the co-culture of 10^4 T cells with 5×10^5 irradiated BALB/c or CAF_1 cells, or with 1×10^4 to 3×10^4 transfected or nontransfected control B-lymphoma cells, for 4 days. Monoclonal antibody to I-A^k (10.2.16) in ascites form was used at a final concentration of 1:800 to block stimulation. For the last 16 hours the cultures were treated with [^{3}H]thymidine (New England Nuclear) (1 μCi per well) and then harvested with a multiharvester. Results are expressed in counts per minute (mean) for triplicate cultures. The standard deviation was less than 15 percent. N.T., not tested.

Irradiated stimulators	I-A haplotype	Stimulation by transfected cells (count/min)			
		Experiment 1		Experiment 2	
		No antibody	With antibody to I-A^k	No antibody	With antibody to I-A^k
BALB/c spleen cells	$\alpha^d\beta^d$	4,170	N.T.	6,283	N.T.
CAF_1 spleen cells	$\alpha^{d/k}\beta^{d/k}$	45,680	26,217	52,905	41,752
TA3 B hybridoma	$\alpha^{d/k}\beta^{d/k}$	26,680	17,263	53,432	26,675
M12.4.1 B lymphoma	$\alpha^d\beta^d$	12,098	11,322	14,331	N.T.
M12.βk3	$\alpha^d\beta^d + \beta^k$	32,222	13,980	41,778	14,232
A20.2J B lymphoma	$\alpha^d\beta^d$	< 0*	< 0	10,805	N.T.
A20.βk20	$\alpha^d\beta^d + \beta^k$	37,748	9,837	46,910	20,383

*The value for the irradiated lymphoma cells alone was subtracted from the actual value in each experiment.

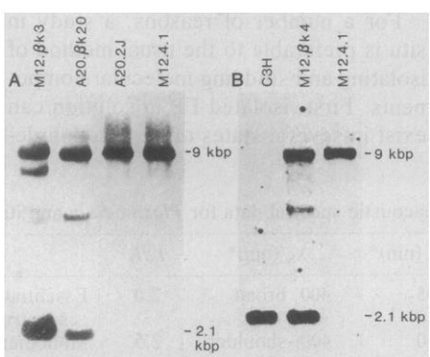

Fig. 3. Transfected B cells containing the β chain were identified by Southern blot analysis (*13*). M12.βk3 cells are M12.4.1 cells that were co-transfected with λ I-Aβ^k and pMSV-neo DNA's. M12.βk4 cells are M12.4.1 cells that were transfected with pβk-neo DNA. A20.βk20 cells are A20.2J B cells that were transfected with pβk-neo (Fig. 1C). DNA (10 μg) from transfected and control cells and from the livers of C3H mice was digested with Bam HI, subjected to electrophoresis in a 1 percent agarose gel, transferred to a nitrocellulose filter, and hybridized to ^{32}P-labeled, 2.1-kilobase Bam HI–Bam HI DNA fragments isolated from λ I-Aβ^k (Fig. 1).

The introduction and functional expression of class II genes constitute an important step in studying the interaction of class II antigens with foreign antigens and with antigen-specific T lymphocytes. The failure of M12.βk3 and A20.βk20 cells, which express a unique α^d/β^k hybrid antigen, to stimulate T cells specific for various antigens in association with the I-A^k molecule may indicate that a T cell that recognizes antigens in association with a particular F_1 hybrid I-A$^{k/d}$ restriction element is required to demonstrate the antigen-presenting function of the transfected gene. Indeed, preliminary results indicate that I-A^d B-lymphoma cells transfected with both the α and β genes of the I-A^b antigen are potent antigen-presenting cells for a panel of T-cell hybridomas specific for various antigens in association with the I-A^b molecule.

Note added in proof: Similar results have also been obtained by Germain *et al.* (*14a*).

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References and Notes

1. J. Klein, A. Juretic, C. N. Baxeranis, Z. A. Nagy, *Nature (London)* **291**, 455 (1981).
2. B. Benacerraf, *Science* **212**, 1229 (1981).
3. The murine I-Aβ chain is a polymorphic subunit of the I-A antigen. The particular I-A allele is indicated by the superscript.
4. J. G. Woodward, A. Orn, R. C. Harmon, R. S. Goodenow, L. Hood, J. A. Frelinger, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3613 (1982).
5. D. H. Margulies *et al.*, *J. Immunol.* **130**, 463 (1970).
6. C. Rabourdin-Combe and B. Mach, *Nature (London)* **303**, 670 (1983).
7. B. Malissen, M. Steinmetz, M. McMillan, M. Pierres, L. Hood, *ibid.* **305**, 440 (1983).
8. K. J. Kim, C. Kanellopoulos-Langevin, R. M. Merwin, D. H. Sachs, R. Asofsky, *J. Immunol.* **122**, 549 (1979).
9. L. M. Glimcher, K. J. Kim, I. Green, W. E. Paul, *J. Exp. Med.* **155**, 445 (1982).
10. M. Wigler, A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, L. Chassin, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1376 (1979).
11. M. Pierres, C. Devaux, M. Dosseto, S. Marchetto, *Immunogenetics* **14**, 481 (1981).
12. J. M. Kupinski, M. L. Plunkett, J. H. Freed, *J. Immunol.* **130**, 2277 (1983).
13. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982).
14. M. Kimoto and G. C. Fathman, *J. Exp. Med.* **152**, 759 (1980).
14a. R. N. Germain, M. A. Norcross, D. H. Margulies, *Nature (London)* **306**, 190 (1983).
15. E. Choi, K. McIntyre, R. N. Germain, J. G. Seidman, *Science* **221**, 283 (1983).
16. M. Malissen, T. Hunkapiller, L. Hood. *ibid.*, p. 750.
17. D. Larhammar *et al.*, *Cell* **34**, 179 (1983).
18. R. Mann, R. C. Mulligan, D. Baltimore, *ibid.* **33**, 153 (1983). Subsequently modified to replace the *gpt* gene with the neomycin-resistant gene by R. C. Mulligan (unpublished).
19. L. H. Glimcher *et al.*, *J. Immunol.* **130**, 2287 (1983).
20. V. T. Oi, P. P. Jones, J. W. Goding, L. A. Herzenberg, *Curr. Top. Microbiol. Immunol.* **81**, 115 (1978).

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The Malaria Parasite Monitored by Photoacoustic Spectroscopy

Abstract. *Noninvasive photoacoustic spectroscopy was used to study the malaria parasites* Plasmodium chabaudi *and* Plasmodium berghei, *their pigment, and ferriprotoporphyrin IX, which is a by-product of the hemoglobin that the parasite ingests. The results indicate that the pigment consists of ferriprotophorphyrin self-aggregates and a noncovalent complex of ferriprotoporphyrin and protein. Spectra of chloroquine-treated parasites reveal in situ interaction between the drug and ferriprotoporphyrin. Chloroquine-resistant parasites, readily distinguishable by this method, appear to degrade hemoglobin only partially.*

Malaria parasites of the genus *Plasmodium* infect erythrocytes, degrade the hemoglobin there for its metabolism, and store the residual ferriprotoporphyrin IX (FP) in a protein-sequestered form termed malaria pigment or hemozoin (*1*). It has been suggested that free hemin (FP chloride) and a FP-chloroquine complex thought to be formed when this drug is added to the system are toxic to the parasite since they lyse cell membranes (*2*). It is thus of interest to monitor the states in which FP exists in the parasite, observe the changes that it undergoes on drug addition, detect any formation of complexes between antimalarial drugs and FP in the parasite, and investigate any differences between drug-sensitive and -resistant parasite strains.

For a number of reasons, a study in situ is preferable to the usual method of isolating and studying molecular components. First, isolated FP in solution can exist in several states of aggregation depending on the solution conditions (*3, 4*), while the interest here is on its status in vivo. Second, FP appears to complex easily with proteins (*5, 6*), a situation that is more likely to occur in the microorganism. Third, the particulate malaria pigment appears to be a heterogeneous population of molecules and complexes that are not easily studied in solution due to solubility and stability problems. Finally, direct comparisons between drug-treated and untreated parasites and between drug-sensitive and -resistant strains are more meaningfully done in situ. We therefore used photoacoustic spectroscopy, a noninvasive technique particularly suitable for condensed phase samples, in order to establish the nature of the malaria pigment, provide direct evidence for FP-drug interactions in the parasite, and distinguish drug-sensitive strains of the microorganisms from resistant ones—a method of potential diagnostic value.

The principles of photoacoustic spectroscopy and its applications in biology have been described elsewhere (*7–10*). The materials for our study were obtained by standard procedures (*1, 2, 11*). Parasite-infected mouse erythrocytes were incubated in Fitch medium for 30 minutes with or without chloroquine. The parasites were then prepared by saponin lysis and N_2 decompression of the infected erythrocytes (*12*). Samples analyzed were lyophilized powders suitably diluted. Since it is not certain whether all the parasites were intact, the spectra represent conditions in situ rather than in vivo. The spectral parameters of interest are the wavelength maxima of the band in the "red" region (λ_r) and the Soret band (λ_S) of the constituent hemin, their relative intensity ratio I_S/I_r (an internally normalized parameter that characterizes each system studied), band-

Table 1. Photoacoustic spectral data for *Plasmodium* and its components.

Sample	λ_r (nm)*	λ_S (nm)*	I_S/I_r	Remarks
1. Hemin (FP chloride), solid	645	400, broad	2.0	Essentially aggregated; resembles the optical absorption spectrum of the aggregates in solution (*3, 5*)
2. Hemozoin isolated from the parasite	630	400, shoulder at 370	2.5	Monomeric FP indicated
3. Hemin and BSA complex, lyophilized	630	400, shoulder at 370	2.5	Monomeric FP; resembles optical spectrum of the complex in solution (*5, 6*)
4. *P. chabaudi*, isolated and lyophilized; chloroquine-sensitive strain	645, 630	400, minor band ~ 430	2.1	Both monomeric and aggregated heme indicated; 430-nm band suggestive of peptide-coordinated heme (*20, 10*)
5. *P. chabaudi*, chloroquine-resistant strain	645, 630	430	1.3	Only peptide-coordinated heme indicated
6. *P. berghei*, chloroquine-sensitive strain	645, 635	400	2.3	I_S/I_r somewhat higher than in case 4; different parasite strain (also uncloned)
7. *P. berghei*, chloroquine-sensitive and treated with chloroquine	640	370	3.0	Blue-shifted Soret band; FP-drug interaction indicated
8. *P. berghei*, chloroquine-sensitive and treated with mefloquine	640	375	2.5	Blue-shifted Soret band; FP-drug interaction indicated

*Error, ±3 nm.