cells from each transformed line was estimated from titration experiments in which graded doses of each cell line were tested for growth in normal mice. The appropriate dose of cells was suspended in 0.1 ml of Hanks balanced salt solution and injected subcutaneously.

Recipient animals were specific pathogen–free female mice of the inbred strain C3H/HeN(MTV$^-$), obtained from the Frederick Cancer Research Facility. Immunosuppression of one group of animals was achieved by thymectomy at 4 weeks of age, followed by the administration of 450 R of whole-body x-radiation 24 hours before the inoculation of transformed cells. Mice in a second group were shaved weekly and exposed to UV radiation from Westinghouse FS40 sunlamps at an average cosine-corrected incident dose rate of 4 J/m^2-sec for 1 hour three times per week, beginning at 8 weeks of age. Approximately 80 percent of the radiation emitted by these lamps is composed of wavelengths between 280 and 340 nm. The mice were irradiated for a minimum of 12 weeks before being used in the experiments. A third (control) group of mice, from the same age-matched pool of mice as the UV-irradiated animals, was not treated. After being injected with the transformed cells, the animals were examined weekly for tumor formation at the injection site for 6 to 9 months or until they died.

Injection of 1×10^8 cells of the parent 10T 1/2 line did not produce tumors in any of the recipient mice, whereas all the lines transformed in vitro produced tumors (Table 1). At a minimum tumorigenic dose, all these cell lines grew in a larger proportion of immunosuppressed than untreated mice, implying that all the lines exhibited some degree of antigenicity. However, only the cell lines produced by transformation in vitro by UV radiation also grew more efficiently in the UV-irradiated mice.

To ascertain whether the preferential development in UV-irradiated mice of cells transformed in vitro by UV radiation is attributable to suppressor lymphocytes, a cell transfer study was performed. C3H mice that had been lethally x-irradiated (850 R) were reconstituted 24 hours later with 5×10^7 spleen and lymph node cells from normal or UV-irradiated donors, or with a mixture of these populations. These animals were challenged subcutaneously 24 hours later with cells from a fibrosarcoma induced in a C3H mouse by repeated sunlamp irradiation (UV-2240), with 254-nm UV radiation–transformed 10T 1/2 cells (TU2), or with MCA-transformed 10T 1/2 cells (MCA5). Lymphoid cells from the UV-irradiated donors could not mediate the rejection of either UV radiation–induced tumor, and they reduced the ability of normal lymphoid cells to effect rejection (Table 2). In contrast, the MCA-transformed 10T 1/2 cell line grew similarly in the three groups of mice, indicating that its growth was unaffected by the presence of the UV radiation–induced suppressor lymphocytes. The experiment was repeated by using a different 10T 1/2 cell line transformed in vitro with UV radiation (TU3), with the same results.

These findings indicate that cells transformed in vitro by UV radiation have an antigenic determinant that is the same as, or similar to, one that is otherwise found only in skin cancers induced in vivo by UV radiation and that is recognized by the antigen-specific, UV radiation–induced suppressor lymphocytes. Thus, this antigenic determinant is related in some way to the action of the carcinogen and is not a unique property of the particular cells transformed in vivo by UV radiation. It is not known whether this determinant is associated with the transformation event itself or whether it results from some heritable effect of UV radiation on the target cells. Nonetheless, it is interesting that this same change is produced in cells after exposure to either the shorter UV wavelength (254 nm) or to wavelengths in the mid-UV range (280 to 320 nm). One question that has arisen concerning in vitro transformation by 254-nm radiation is its relevance to the induction of skin cancers in mice by repeated exposure to mid-UV radiation. Our finding that both a single exposure of fibroblasts to 254-nm radiation in vitro and repeated irradiation of mouse skin with mid-UV wavelengths induce the same antigenic determinant supports the validity of the in vitro transformation system as a model for UV radiation carcinogenesis.

MICHAEL S. FISHER
MARGARET L. KRIPKE*
Basic Research Program–LBI, Frederick Cancer Research Facility, Frederick, Maryland 21701
GERALD L. CHAN
Laboratory of Radiobiology, Harvard School of Public Health, Boston, Massachusetts 02115

References and Notes

1. M. L. Kripke, *J. Natl. Cancer Inst.* **53**, 1333 (1974); *Cancer Res.* **37**, (1977); _____ and M. S. Fisher, *J. Natl. Cancer Inst.* **57**, 211 (1976).
2. M. S. Fisher and M. L. Kripke, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1688 (1977); C. W. Spellman and R. A. Daynes, *Transplantation* **24**, 120 (1977); M. S. Fisher and M. L. Kripke, *Science* **216**, 1133 (1982).
3. M. S. Fisher and M. L. Kripke, *J. Immunol.* **121**, 1139 (1978); M. L. Kripke *et al.*, *Transplantation* **28**, 212 (1979); M. L. Kripke *et al.*, *J. Natl. Cancer Inst.* **68**, 685 (1982).
4. C. A. Reznikoff, J. S. Bertram, D. W. Brankow, C. Heidelberger, *Cancer Res.* **33**, 3239 (1973).
5. G. L. Chan and J. B. Little, *Nature (London)* **264**, 442 (1976).
6. Research sponsored by the National Cancer Institute under contract N01-CO-23909 with Litton Bionetics, Inc., and by NIH grants CA-09078 and CA-11751.
* Present address: Department of Immunology, Box HMB 178, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

28 October 1983; accepted 21 December 1983

Adaptive Response of Human Lymphocytes to Low Concentrations of Radioactive Thymidine

Abstract. *When human lymphocytes were cultured with [^{3}H]thymidine, which acts as a source of low-level chronic radiation, and then exposed to 150 rad of x-rays at 5, 7, 9, or 11 hours before fixation, the yield of chromatid aberrations was less than the sum of the yields of aberrations induced by [^{3}H]thymidine and x-rays separately. Often fewer aberrations were found after exposure to radiation from both sources than were found after exposure to x-rays alone. At the same fixation times, nonradioactive thymidine did not affect the yield of x-ray-induced aberrations. The same phenomenon occurred at earlier fixation times, after exposure to 30 or 40 rad of x-rays and [^{3}H]thymidine. This response is analogous to the adaptive response to alkylating agents whereby prior treatment with small doses for a long period reduces the damage occurring from large doses of similar agents given for a short time.*

Low doses of radiation have frequently been reported to affect the response of cells to subsequent doses (*1*), but this phenomenon is still a matter of controversy [for example, see (*2*)]. Incorporation of [^{3}H]thymidine ([^{3}H]dThd) into chromosomes causes various kinds of genetic damage, including chromatid aberrations (*3*), which also can be induced by x-rays. To study the interaction of low chronic doses of radiation with subsequent higher acute doses in the production of chromatid aberrations, we carried out experiments in which human peripheral lymphocytes in culture were first labeled with [^{3}H]dThd and then exposed to x-rays 3 to 11 hours before fixation. Radiation from incorporated

isotopes seems to evoke a response that is reminiscent of the adaptive response found for alkylating agents (*4*); that is, low levels of chronic radiation can trigger or induce increased repair of radiation-induced chromosome breaks.

Heparinized whole blood was obtained by venipuncture from healthy male adults. Microcultures containing 0.5 ml of blood in 5 ml of RPMI 1640 medium containing 10 percent fetal calf serum, 200 m*M* glutamine, and 2 percent phytohemagglutinin M (Gibco) were established. The medium also contained penicillin (100 I.U./ml) and streptomycin (100 μg/ml). Because RPMI 1640 medium does not contain thymidine, under our standard conditions the only thymidine present was the small amount in the whole blood and in the fetal calf serum (*5*).

Either [^{3}H]dThd (0.01 to 0.1 μCi/ml; specific activity, 6.7 Ci/ml) or nonradioactive thymidine was added to the experimental cultures 4 to 6 hours after the cultures were established. Some cultures also contained 5-bromodeoxyuridine (BrdUrd) (20 μ*M* final concentration) so that the number of times a cell divided in culture could be determined in harlequin-stained preparations (*6*). On the second or third day of culture the cells were irradiated with 300-kilovolt peak (kVp) x-rays (General Electric Maxitron; half-value layer, 2 mm of Cu, 150 rad/min) (Tables 1 to 3), or 180-kVp x-rays (Gilardoni T/200/6; 4 mm of Al added filtration, 120 rad/min) (Table 4). Depending on the experiment, cells were fixed from 3 to 11 hours after irradiation. Two or 3 hours before fixation, 0.1 ml of Colcemid (final concentration, 2×10^{-7} *M*) was added to each culture. Fixation was performed according to standard cytologic procedures; that is, the cells were exposed to a hypotonic solution and then fixed in a mixture of methanol and acetic acid (3:1), and the slides were stained with Giemsa. In the experiments with BrdUrd, the slides were stained by a modification of the fluorescence-plus-Giemsa method (*7*), so that only cells in their first division in culture were scored [see (*8*)]. In some experiments, autoradiographs of cells exposed to [^{3}H]dThd were prepared by dipping the slides into Ilford L4 emulsion.

The first experiments were carried out to see whether exposure of lymphocytes to continuous low doses of radiation from incorporated radioisotopes would affect the cells' response to subsequent doses of x-rays. When cells that had incorporated [^{3}H]dThd were fixed 7 hours after receiving 150 rad of x-radiation, the yield of chromatid and isochromatid deletions was significantly less than the sum of the numbers of aberrations induced by [^{3}H]dThd and x-rays administered separately (Table 1). In fact, in these three experiments, combined exposure to [^{3}H]dThd and x-rays caused even fewer aberrations than were induced by x-rays alone.

Table 1. Effects of continuous treatment with [^{3}H]dThd on the frequency of chromatid aberrations obtained in human lymphocytes treated with 150 rad of x-rays 46 hours after stimulation and fixed 7 hours later. Cultures were harvested after 2 hours of exposure to Colcemid. In experiment 3, 20 μ*M* BrdUrd was added 8 hours after stimulation. One hundred cells (first-division metaphases in experiment 3) were scored for each point.

Treatment	Gaps	Chromatid and isochromatid deletions	Expected number of deletions	Chromatid exchanges	Proportion of metaphases in each division	
					First	Second and third
Experiment 1						
None	3	0		0		
[^{3}H]dThd (0.01 μCi/ml)	3	2		0		
[^{3}H]dThd (0.1 μCi/ml)	6	5		0		
150 rad	12	36		1		
[^{3}H]dThd (0.01 μCi/ml) + 150 rad	9	23	38	3		
[^{3}H]dThd (0.1 μCi/ml) + 150 rad	10	13*	41	3		
Experiment 2						
None	7	1		0		
[^{3}H]dThd (0.1 μCi/ml)	19	25		0		
150 rad	17	47		1		
[^{3}H]dThd (0.1 μCi/ml) + 150 rad	14	38*	71	1		
Experiment 3 (BrdUrd added)						
None	4	2		0	55	45
[^{3}H]dThd (0.1 μCi/ml)	5	7		0	41	59
150 rad	6	33		1	86	14
[^{3}H]dThd (0.1 μCi/ml) + 150 rad	9	20*	38	2	78	22

*Difference from the expected frequency (the sum of the yields induced by x-rays alone, induced by [^{3}H]dThd alone, and the control value) at $P < 0.01$ (Student's *t*-test).

Table 2. Effects of continuous treatment with [^{3}H]dThd on the frequency of chromatid aberrations obtained in human lymphocytes treated with 40 rad of x-rays 50 hours after stimulation and fixed 3 hours later. Cultures were harvested after 3 hours of exposure to Colcemid; 20 μ*M* BrdUrd was added 8 hours after stimulation. One hundred first-division metaphases were scored for each point.

Treatment	Gaps	Chromatid and isochromatid deletions	Expected number of deletions	Chromatid exchanges	Proportion of metaphases in each division	
					First	Second and third
None	3				65	35
[^{3}H]dThd (0.01 μCi/ml)	4	1			67	33
[^{3}H]dThd (0.1 μCi/ml)	10	11			72	28
40 rad	9	58		3	67	33
[^{3}H]dThd (0.01 μCi/ml) + 40 rad	10	37†	59	1	78	22
[^{3}H]dThd (0.1 μCi/ml) + 40 rad	16	34*	69	2	80	20

*Difference from the expected frequency (the sum of the yields induced by x-rays alone, induced by [^{3}H]dThd alone, and the control value) at $P < 0.01$ (Student's *t*-test). †$P < 0.05$.

Because the number of aberrations decreases as cells containing the aberrations divide (*9*), an experiment was carried out to see if the decline in aberration number might be an artifact caused by the scoring of cells that had divided more than once (Table 1). In this experiment the cells were exposed to 20 μM BrdUrd as well as [^{3}H]dThd so that we could restrict the scoring of aberrations to cells that were dividing for the first time in culture. The results show that the observed decline in aberration number was not caused by the loss of aberrations through cell division.

In previous experiments with plant cells, combined exposures to far-red light and x-rays showed that the number of chromatid aberrations found at any given time after irradiation was merely a reflection of a differential sensitivity of the cells in different parts of the G_2 phase of the cell cycle (*10*). When combined treatments were given, cell progression to metaphase, the stage at which aberrations are scored, was slower than the progression in cells that had been treated only with x-rays. This led to comparisons between cell populations of differing sensitivity. That a difference in cell-stage sensitivity might also occur in human lymphocyte cultures was suggested in experiments in which the cells were fixed only 3 hours after irradiation (Table 2). In this instance, a dose of 40 rad induced more chromatid aberrations than were found after a dose of 150 rad in cells fixed at later times (*11*).

The data in Table 2 also show that exposure to [^{3}H]dThd plus 40 rad of x-rays led to fewer aberrations than would be expected in cells fixed only 3 hours after irradiation. As with the cells exposed to 150 rad of x-rays and fixed 7 hours later, this effect occurred even when the amount of [^{3}H]dThd was reduced by a factor of 10. In addition, experiments carried out in the presence of BrdUrd showed that, although 40 rad of x-rays did not affect the proliferation of cells (Table 2), when x-rays and 0.1 μCi/ml of [^{3}H]dThd were combined, more of the cells in the culture were in their first division and concomitantly fewer had divided two or three times.

That differential stage sensitivity does not account for the decline in aberration numbers, however, is shown in experiments in which the cells were successively fixed at 5, 7, 9, or 11 hours after x-ray treatment (Table 3). Although in this set of experiments the yield of chromatid breaks after combined treatment was not smaller than that after x-rays alone (as it was in the experiments shown in Table 1), at all doses the observed yields were smaller than would be expected from the sum of the yields induced by x-rays and [^{3}H]dThd separately. Furthermore, although 150 rad by itself delayed cell proliferation in cultures (Table 1), as found by Purrott *et al.* (*12*) after irradiation during the G_1 stage, the additional presence of [^{3}H]dThd did not cause a further slowing of the cell cycle (Table 1). These results show that, unlike the situation that prevails after far-red light and x-ray exposure in plants, the decline in chromatid breaks in human lymphocytes is not a mere reflection of stage sensitivity coupled with a differential delay in the progression of irradiated cells to metaphase.

Because RPMI 1640 medium contains no thymidine, control experiments were performed with small amounts of nonradioactive thymidine at approximately the same concentrations as those found in the radioisotope mixture, which are close to the physiologic concentrations of the nucleotide (*13*). These concentrations do not induce aberrations (*14*) (Table 4). The results showed that nonradioactive thymidine did not affect the response to x-rays in cells exposed to 150 rad and fixed 6 hours later (Table 4).

Since the cells were grown in medium that was deficient in thymidine, it seemed possible that perturbations in the nucleotide pools, which have profound effects [for review see (*15*)], could have influenced the results. Inhibition of thymidylate synthetase by 5-fluorodeoxyuridine can lead to broken chromatids (*16*), and growth of cells in medium lacking folic acid and thymidine can produce

Table 3. Effects of continuous treatment with [^{3}H]dThd on the frequency of chromatid aberrations obtained in human lymphocytes treated with 150 rad of x-rays 46 hours after stimulation and fixed 5 to 11 hours later. One hundred cells were scored for each point.

Treatment	Fixation time after irradiation	Gaps	Chromatid and isochromatid deletions	Expected number of deletions	Chromatid exchanges
None	5	6	1		0
[^{3}H]dThd (0.1 μCi/ml)	5	9	25		0
150 rad	5	7	28		3
[^{3}H]dThd (0.1 μCi/ml) + 150 rad	5	11	30*	52	2
150 rad	7	6	22		
[^{3}H]dThd (0.1 μCi/ml) + 150 rad	7	6	22*	43.5	1
150 rad	9	13	27		4
[^{3}H]dThd (0.1 μCi/ml) + 150 rad	9	19	34	48.5	3
None	11		2		
[^{3}H]dThd (0.1 μCi/ml)	11	9	21		
150 rad	11	9	30		1
[^{3}H]dThd (0.1 μCi/ml) + 150 rad	11	10	33†	49	2

*Difference from the expected frequency (the sum of the yields induced by x-rays alone, the mean yield induced by [^{3}H]dThd alone, and the control value) at $P < 0.01$ (Student's t-test). †$P < 0.05$.

Table 4. Effects of continuous treatment with [^{3}H]dThd or dThd on the frequency of chromatid aberrations obtained in human lymphocytes with 150 rad of x-rays 48 hours after stimulation and fixed 6 hours later. Two hundred cells were scored for each point except where indicated otherwise.

Treatment	Gaps	Chromatid and isochromatid deletions	Expected number of deletions	Chromatid exchanges
Experiment 1				
None*	2	2		0
dThd ($1.5 \times 10^{-7} M$)*	1	2		0
[^{3}H]dThd (0.1 μCi/ml)	8	36		0
150 rad	14	50		3
dThd ($1.5 \times 10^{-7} M$) + 150 rad	15	42	50	7
dThd ($1.5 \times 10^{-8} M$) + 150 rad	14	40	50	7
[^{3}H]dThd (0.1 μCi/ml) + 150 rad	18	59†	82	4
Experiment 2				
None*	3	1		0
dThd ($1.5 \times 10^{-8} M$)	1	3		0
150 rad	20	36		1
dThd ($1.5 \times 10^{-8} M$) + 150 rad	17	35	37	

*One hundred cells were scored. †Difference from the expected frequency (the sum of the yields induced by x-rays alone, the mean yield induced by [^{3}H]dThd alone, and the control value) at $P < 0.05$ (one-tailed Student's t-test).

micronuclei that are the result of broken chromosomes (*17*). That the present results are not attributable to perturbations in nucleotide pools, however, can be deduced from the fact that only the radioactive form of thymidine led to the effect, even though both radioactive and nonradioactive thymidine were present at the same concentration. Furthermore, the labeling patterns of the chromosomes in the autoradiographs prepared from cells cultured in the presence of [^{3}H]dThd indicated that the [^{3}H]dThd was completely used in the first cell cycle, so that in all cases the second cycle was passed in the absence of exogenous thymidine, and all of the cells should have been subjected to the same imbalances. Thus, the phenomenon is most likely caused by the radiation from the tritium.

The major effect observed in this study [see also (*18*)] was that radiation from incorporated isotopes seems to trigger a response that is analogous to the adaptive response to alkylating agents reported by Samson and Schwartz (*4*). In their experiments, cells chronically exposed to small doses of an alkylating agent were resistant to subsequent exposures to large acute doses of the same chemical. The response was not related to differences in cell cycling. In the present experiments, in which cells were given 150 rad of x-rays after exposure to chronic radiation from an incorporated radioisotope, the response was similar to the adaptive response, but now it occurred for ionizing radiation–induced chromatid aberrations in human lymphocytes. The results are consistent with the concept that exposure to low levels of chronic radiation can trigger or induce increased repair of radiation-induced chromosome breaks.

This putative radiation-stimulated repair is reminiscent of the SOS repair frequently reported to be induced in bacterial systems (*19*), although SOS repair leads to more, not fewer, mutations. Furthermore, because hydrogen peroxide and ionizing radiation yield similar reactive species, Demple and Halbrook (*20*) tested the effect of prior treatment with H_2O_2 on the resistance of *Escherichia coli* to x-rays. They concluded that peroxide induces the repair of lesions induced by peroxide itself and by ionizing radiation. In *Drosophila melanogaster*, x-rays themselves have been reported to induce DNA repair and thus reduce mitotic recombination (*21*).

Perhaps of more specific interest are reports that the activity of the enzyme poly(ADP-ribose) polymerase (ADP, adenosine diphosphate), which has been involved in repair, is increased by DNA strand breaks of the type produced by chronic radiation from incorporated [^{3}H]dThd (*22*). However, whether the induction of this enzyme is related to the adaptive response observed in the present experiments is unknown.

GREGORIO OLIVIERI
JUDY BODYCOTE
SHELDON WOLFF

Laboratory of Radiobiology and Environmental Health, University of California, San Francisco 94143

References and Notes

1. G. R. Lane, *Heredity* **5**, 1 (1951); T. D. Luckey, *Hormesis with Ionizing Radiation* (CRC Press, Boca Raton, Fla., 1980), pp. 1–222.
2. F. J. De Serres and N. H. Giles, *Genetics* **38**, 407 (1953).
3. M. A Bender, P. C. Gooch, D. M. Prescott, *Cytogenetics* **1**, 65 (1962); R. R. Klevecz and T. C. Hsu, *Proc. Natl. Acad. Sci. U.S.A.* **52**, 811 (1964); C. H. Ockey, *Int. J. Radiat. Biol.* **13**, 479 (1967); B. K. Vig, S. B. Kontras, E. F. Paddock, *Cytogenetics* **7**, 189 (1968); C. R. Geard, *Radiat. Res.* **61**, 118 (1975); T.-A. Hori and S. Nakai, *Mutat. Res.* **50**, 101 (1978).
4. L. Samson and J. L. Schwartz, in *Induced Mutagenesis*, C. W. Lawrence, Ed. (Plenum, New York, 1983).
5. J. C. Schaer *et al.*, *Exp. Cell Biol.* **46**, 1 (1978).
6. R. Tice, E. L. Schneider, J. M. Rary, *Exp. Cell Res.* **102**, 232 (1976).
7. P. Perry and S. Wolff, *Nature (London)* **251**, 156 (1974).
8. K. Morimoto and S. Wolff, *ibid.* **288**, 604 (1980).
9. A. V. Carrano and J. A. Heddle, *J. Theor. Biol.* **38**, 289 (1973).
10. S. Wolff and H. E. Luippold, *Photochem. Photobiol.* **4**, 439 (1965).
11. G. Olivieri and A. Micheli, *Mutat. Res.* **122**, 64 (1983).
12. R. J. Purrott, N. Vulpis, D. C. Lloyd, *ibid.* **69**, 275 (1980).
13. L. Holden, A. V. Hoffbrand, M. H. N. Tattersall, *Eur. J. Cancer* **16**, 115 (1980).
14. G. Olivieri, J. Bodycote, S. Wolff, unpublished data.
15. B. A. Kunz, *Environ. Mutagen.* **4**, 695 (1982).
16. J. H. Taylor, W. F. Haut, J. Tung, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 190 (1962); S. Bell and S. Wolff, *ibid.* **51**, 195 (1964).
17. P. B. Jacky, B. Beek, G. R. Sutherland, *Science* **220**, 69 (1983).
18. J. Pohl-Ruling and P. Fischer, *Radiat. Res.* **80**, 61 (1979); H. Tuschl, H. Altmann, R. Kovac, A. Topaloglou, D. Egg, R. Gunther, *ibid.* **81**, 1 (1980).
19. B. Bridges and A. Lehman, *Nature (London)* **298**, 118 (1980).
20. B. Demple and J. Halbrook, *ibid.* **304**, 466 (1983).
21. J. A. Kennison and P. Ripoll, *Genetics* **98**, 91 (1981).
22. R. C. Benjamin and D. M. Gill, *J. Biol. Chem.* **255**, 1049 (1980).
23. This work was supported by U.S. Department of Energy contract DE-AM03-76-SF01012, and by Euratom contracts BIO-E-400 and BIO-E-450 (to G.O.).

17 October 1983; accepted 14 December 1983

Identification of an Erythrocyte Component Carrying the Duffy Blood Group Fy^a Antigen

Abstract. *The erythrocyte component carrying the Duffy blood group antigen Fy^a has been identified as a 35- to 43-kilodalton protein. The protein is degraded by proteases, chymotrypsin, and Pronase, which destroy its antigenicity on intact erythrocytes. Its unusual property of aggregating on being boiled in 5 percent sodium dodecyl sulfate with 5 percent 2-mercaptoethanol distinguishes it from other erythrocyte membrane proteins described to date.*

The Duffy blood group system consists of four major phenotypes, Fy(a+ b−), Fy(a− b+), Fy(a+ b+), and Fy(a− b−) (*1*). The system is defined by two antisera, anti-Fy^a and anti-Fy^b; Duffy negative erythrocytes, Fy(a− b−), are not agglutinated by either antiserum. With rare exceptions, the Duffy negative phenotype occurs only in blacks. Blacks with Duffy negative erythrocytes cannot be infected by the human malaria parasite *Plasmodium vivax* (*2*). Duffy negative human erythrocytes are resistant to invasion in vitro by a monkey malaria parasite, *Plasmodium knowlesi*, that can invade Duffy positive human erythrocytes (*3*). Anti-Fy^a and anti-Fy^b sera block invasion of Fy(a+ b−) and Fy(a− b+) erythrocytes, respectively (*3, 4*). These observations suggest that molecules containing the Duffy determinants are involved in the invasion process by these malarias.

Data on the molecular nature of the Duffy antigens are limited and inconclusive (*5*). In the present study we combined the high resolution of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with protein blotting or electroelution in order to identify an erythrocyte membrane component that carries Fy^a determinants. The component, which has an apparent molecular weight of 35,000 to 43,000, appears to be different from previously described erythrocyte components.

Erythrocyte ghosts were prepared from Fy(a+ b−), Fy(a− b+), and Fy(a− b−) erythrocytes and extracted in 5 percent (weight to volume) SDS and 5 percent (by volume) 2-mercaptoethanol. Components of each extract were separated by SDS-PAGE and electrophoretically transferred onto nitrocellulose paper, which was then reacted with human serum containing anti-Fy^a specificity (*6, 7*). Iodine-125–labeled protein A (Amersham) was used as a probe to locate antigen-antibody complexes on the paper.

Anti-Fy^a reacted specifically with an erythrocyte membrane component mi-