Analysis of RNA from sensitive and resistant cell lines further supported our conclusion that MT gene expression is increased in drug-resistant cells. Total RNA probed with a 3.0-kb Hind III fragment of human genomic DNA containing MT-II_A (*14*) revealed an increase of MT mRNA in the L1210/CP (tenfold) (Fig. 1A), SCC-25/CP (sixfold) (Fig. 1, B and C), and A-253/CP (13.5-fold) cells. Neither L1210/DACH (Fig. 1A) nor L1210/CP-R showed any increase in MT mRNA. No evidence for MT gene amplification could be seen in SCC-25/CP or L1210/CP cells by DNA blot analysis, suggesting an enhanced rate of gene transcription or increased mRNA stability as the likely cause of MT overexpression.

Long-term exposure to drugs or heavy metals can have multiple effects on cells (*11*). Therefore, to establish a direct role for MT in the acquisition of resistance to anticancer agents, we transfected mouse C127 cells with a bovine papilloma virus (BPV) shuttle vector containing a human MT-II_A (hMT-II_A) gene construct (*15*). Cells transfected with BPV–hMT-II_A had a greater than tenfold increase in MT compared to cells transfected with BPV alone, as shown by ELISA, and were resistant to CP over a wide range of concentrations (Fig. 2). We observed a 4.4-fold increase in resistance to CP in cells transfected with BPV–hMT-II_A as compared to the cells transfected with the BPV vector alone (Table 2). These cells were equally resistant to melphalan and chlorambucil, displayed only modest resistance to bleomycin and doxorubicin, and showed no significant resistance to 5-fluorouracil and vincristine (Fig. 2 and Table 2). Therefore, MT may confer selective resistance to sulfhydryl-reactive alkylating agents. This possibility is substantiated by our observation that tumor cell lines, such as SCC-25/CP and L1210/CP, with acquired resistance to CP, were also cross-resistant to melphalan and chlorambucil but not to bleomycin.

Our results indicate that increased MT content can make tumor cells resistant to clinically important cancer chemotherapeutic agents such as CP, melphalan, and chlorambucil. We have shown that (i) tumor cells with increased MT content are resistant to both alkylating agents and CP, (ii) cells with acquired resistance to CP frequently have an increase in MT and overexpress MT mRNA, (iii) reversal of the CP-resistance phenotype is accompanied by a decrease in MT content, and (iv) introduction of a eukaryotic expression vector encoding MT into cells confers the drug-resistance phenotype. Although additional mechanisms of resistance to agents such as CP appear to exist (*4, 9*), the role of MT in drug resistance may be extensive. Neoplastic cells may express increased MT owing to the presence of activated Ha-*ras* oncogenes (*15*). Furthermore, various stimuli are capable of inducing cellular MT synthesis. Therapeutic agents such as interferons and steroids, which are commonly used in the treatment of malignant diseases, induce MT (*5*) and may induce transient drug resistance. It remains to be determined whether long-term exposure to CP directly induces MT synthesis or whether the high frequency of overexpression of MT in CP-resistant cells reflects selection. A further understanding of the cause of MT overexpression should facilitate the development of therapeutic approaches to circumvent this mechanism of resistance to antineoplastic drugs.

REFERENCES AND NOTES

1. S. E. Sherman *et al.*, *Science* **230**, 412 (1985); A. Eastman, *Pharmacol. Therap.* **34**, 155 (1987).
2. W. B. Pratt and R. W. Ruddon, *The Anticancer Drugs* (Oxford Univ. Press, New York, 1979).
3. R. F. Borch, in *Metabolism and Action of Anticancer Drugs*, G. Powis and R. Prough, Eds. (Taylor and Francis, London, 1987), pp. 163–193.
4. B. A. Teicher *et al.*, *Cancer Res.* **46**, 4379 (1986); K. J. Scanlon and M. Kashani-Sabet, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 650 (1988); E. Frei III *et al.*, *ibid.* **82**, 2158 (1985).
5. D. Hamer, *Annu. Rev. Biochem.* **55**, 913 (1986); H. E. Rugstad, *Eur. Surg. Res.* **16** (suppl. 2), 102 (1984).
6. L. Endresen *et al.*, *Cancer Res.* **43**, 2918 (1983); A. Bakka, *Toxicol. Appl. Pharmacol.* **61**, 215 (1981).
7. R. A. Tobey *et al.*, *Cancer Res.* **42**, 2980 (1982).
8. A. Kraker, J. Schmidt, S. Krezoski, D. H. Petering, *Biochem. Biophys. Res. Commun.* **130**, 786 (1985); A. Zelazowski, J. Garvey, J. Hoeschele, *Arch. Biochem. Biophys.* **229**, 296 (1984).
9. B. A. Teicher *et al.*, *Cancer Res.* **47**, 388 (1987).
10. P. A. Andrews, M. P. Murphy, S. B. Howell, *Cancer Chemother. Pharmacol.* **19**, 149 (1987).
11. W. Levinson, H. Oppermann, J. Jackson, *Biochim. Biophys. Acta* **606**, 170 (1980); J. C. Seagrave, R. A. Tobey, C. E. Hildebrand, *Biochem. Pharmacol.* **32**, 3017 (1983).
12. M. Waalkes, J. Garvey, C. D. Klaassen, *Toxicol. Appl. Pharmacol.* **79**, 524 (1985); J. S. Garvey, R. J. Vander Mallie, C. C. Chang, *Methods Enzymol.* **84**, 121 (1982).
13. A. Eastman and E. Bresnick, *Biochem. Pharmacol.* **30**, 2721 (1981).
14. D. Durnam and R. Palmiter, *Mol. Cell. Biol.* **4**, 4844 (1984); T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), p. 196.
15. C. J. Schmidt, M. Z. Jubier, D. H. Hamer, *J. Biol. Chem.* **260**, 7731 (1985); C. J. Schmidt and D. H. Hamer, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3346 (1986).
16. G. J. Finlay, B. C. Baguley, W. R. Wilson, *Anal. Biochem.* **139**, 272 (1984); J. Carmichael, W. G. De Graff, A. D. Gazdar, J. D. Minna, J. B. Mitchell, *Cancer Res.* **47**, 936 (1987).
17. J. S. Lazo, W. H. Hait, K. A. Kennedy, I. D. Braun, B. Meandzija, *Mol. Pharmacol.* **27**, 387 (1985).
18. D. Rodbard, P. L. Rayford, J. A. Cooper, G. T. Ross, *J. Clin. Endocrinol.* **28**, 1412 (1968).
19. M. M. Bradford, *Anal. Biochem.* **72**, 248 (1976).
20. A. P. Feinberg and B. Vogelstein, *ibid.* **132**, 6 (1983).

21. We thank S. A. Holden for providing the human tumor cell lines and I. D. Braun for technical assistance. Supported in part by NIH grants CA-43917, CA-01012, CA-38497, CA-01205, and CA-32244, by American Cancer Society grant CH-316, and by the Pharmaceutical Manufacturers Association Foundation.

13 May 1988; accepted 19 July 1988

Induction of B Cell Unresponsiveness to Noninherited Maternal HLA Antigens During Fetal Life

FRANS H. J. CLAAS, YVONNE GIJBELS, JACQUELINE VAN DER VELDEN-DE MUNCK, JON J. VAN ROOD*

Patients who have received many transfusions become highly sensitized and develop antibodies against almost all HLA alloantigens, so that finding a cross-match negative kidney donor is difficult. A survey of those patients showed that 50 percent did not form antibodies against the noninherited maternal HLA antigens. Apart from the obvious clinical implications, the data indicate that a human equivalent of murine neonatal or actively acquired tolerance has now been identified.

ACTIVELY ACQUIRED TOLERANCE IN mice to the antigens of the murine major histocompatibility complex (H-2) is induced by exposure of the animals to allogeneic lymphocytes within 24 hours of birth and often leads to antigen-specific and lifelong immunological nonresponsiveness (*1, 2*). Actively acquired tolerance to the major histocompatibility complex (MHC) in humans (HLA) cannot be studied in the same way. However, we have evidence for the existence of neonatal tolerance in humans in a study of 26 highly sensitized patients waiting for a renal allograft. These patients had developed complement-dependent antibodies to the HLA antigens of almost all unrelated caucasoid donors. It is difficult to find a kidney donor for such patients, as the cross-match with almost all potential donors is positive, ex-

Department of Immunohaematology and Blood Bank, University Hospital, Leiden, the Netherlands.

*To whom correspondence should be addressed.

cept when a potential donor is HLA class I (HLA-A and -B) identical to the patient. These patients constitute about 15% of the combined waiting lists of the organ exchange organizations in Europe (*3*).

Therefore, we identified "permissible mismatches" for highly sensitized patients, by testing their sera against a panel of lymphocytes that were mismatched for only one HLA class I antigen. We found, for 59 out of the first 73 patients tested, HLA class I antigens that, although different from those present in the recipient, did not lead to a positive cross-match. This approach has led to a significant increase in size of the potential kidney donor pool for these patients. We could identify for 34 of the 59 patients an HLA-DR compatible, cross-match negative kidney that carried one or more of the "permissible" class I mismatches. These transplants did very well, with 29 grafts (85%) surviving for 1 year after transplantation (*4*).

We realized later that the permissible mismatches often included those HLA antigens of the patient's mother that the patient had not inherited (not inherited maternal antigen; NIMA). We report our findings and discuss the possible implications for organ transplantation and HLA in relation to disease studies.

We studied 26 patients with end-stage renal disease on the waiting list of Eurotransplant. These patients had antibodies that reacted with the lymphocytes of more than 85% of the members of a panel. This panel (size: 53 individuals) was selected so that all HLA-A and -B antigens were present in at least three donors in different combinations, to determine the specificity of the patients' antibodies. The parents of the patients were HLA typed; in 15 of the 26 patients, the permissible class I mismatches included NIMAs (Table 1). Six of these patients had only one of the two NIMAs (either HLA-A or -B) classified as an acceptable mismatch, suggesting that not all HLA antigens have the same ability to induce or maintain nonresponsiveness. These patients formed antibodies against HLA-A2, -B8, or -B40, which might be due to their strong immunogenicity (*5*). The polymorphism of the HLA system argues against the possibility that our findings are due to chance; 23 allelomorphic genes of the HLA-A locus and 47 of the HLA-B locus have been identified (*6*).

The noninherited paternal haplotypes were analyzed as a control; only 2 of the 25 noninherited paternal HLA-A or -B antigens (NIPA) tested were acceptable mismatches, which emphasized the preferential nonresponsiveness to NIMA ($P < 0.005$ by Fisher's exact test).

The presence of specific alloantibodies against NIMAs and NIPAs was also determined. In the cases where NIMAs and NIPAs were not qualified as acceptable mismatches, specific antibodies to these antigens were found. Such antibodies were more frequently found to NIPAs (23 of 25) than to NIMAs (24 of 45). To ascertain that these findings were not due to a higher degree of cross-reactivity of the patients' HLA antigens with the NIMAs, we counted the actual number of cross-reactive noninherited HLA antigens of the mothers and compared these with the number of cross-reactive noninherited HLA antigens of the fathers. The frequencies turned out to be virtually identical (38 and 37%, respectively) in the group of patients studied.

Although some patients lack antibodies to the noninherited maternal antigens, this does not directly demonstrate that the patients are tolerant to these antigens. We think that the blood transfusion history of the patients indicates that it is very likely that the patients have received transfusions with cells bearing maternal antigens. For instance, patients ZA and BR had received 30 and 50 transfusions, respectively, but were nonetheless nonresponsive to HLA-A1 and -A3, antigens with a phenotype frequency in 505 healthy Dutch blood transfusion donors of 31 and 33%, respectively. The chance that the patients had received only HLA-A1 and HLA-A3 negative blood transfusions is thus equal to $(0.69)^{30}$ for the first and $(0.67)^{50}$ for the second patient ($P < 0.0001$).

One explanation for our results is that during early fetal life maternal cells entering the bloodstream of the fetus might be responsible for the induction of nonresponsiveness. Alternatively, when maternal antigens or cells (or both) enter the fetal bloodstream during later fetal life, immunization can take place. This is in accord with current hypotheses on the development of the immune system during fetal life (*7*). The presence of maternal cells in the fetal bloodstream is supported by the observation that newborn infants form antibodies to noninherited maternal HLA antigens (*8*). Furthermore, evidence has been presented for the existence of actively acquired tolerance to rhesus blood group antigens (*9*).

The assumption that true tolerance is maintained by chimerism (*10*) could explain why some patients had no antibodies to both the HLA-A and -B antigens on the noninherited maternal haplotype. However, chimerism may not account for the cases in which the nonresponsiveness is to just one class I antigen (for example, A1) and not the other (B8; Table 1, patient JO). In these patients an idiotype–anti-idiotype network may play a role in the maintenance of the nonresponsiveness (*11*).

Table 1. Comparison of the NIMA haplotype to the acceptable mismatches of highly sensitized patients. HLA class I typing was done by a standard NIH microcytotoxicity assay with 120 highly selected alloantisera (*17*). Sera were tested for panel reactive antibodies with a complement-dependent cytotoxicity assay. In brief, heat-inactivated serum (1 μl) was added to Ficoll-hypaque–isolated mononuclear cells (4000) and incubated for 30 min at room temperature. Rabbit serum (5 μl) was added, the mixture was incubated for 60 min at room temperature, and 0.03% propidium iodide (5 μl) was added. The test was read on an automated inverted microscope (*18*). The panel reactivity (PR) was determined by testing the sera of patients against a panel of at least 50 lymphocyte donors selected so that all HLA-A and -B antigens were represented. The acceptable mismatches, that is, the HLA-A or -B alloantigens toward which the patient had not formed antibodies, were determined by testing the patient's serum against a panel of lymphocyte donors, who were mismatched for only one HLA class I antigen (*4*). In 11 patients the acceptable mismatches did not include the antigens present on the not inherited maternal haplotype.

Patients	PR (%)*	Noninherited maternal haplotype	Acceptable mismatches include:
MI	100	A11 B35	A11 B35
JO	97	A1 B8	A1 †
EN	95	A30 B35	A30 B35
VE	100	‡ B7	B7
KO	96	A3 B35	A3 B35
ST	100	A2 B27	† B27
ZA	90	A1 B8	A1 †
BO	98	A26 B40	A26 B40
BR	100	A3 B40	A3 †
CA	98	A28 B44	A28 B44
NO	89	A2 B40	† B40
MO	98	‡ B35	B35
VM	98	A32 B27	A32 B27
KA	100	A2 B18	† B18
VG	100	‡ B40	B40

*PR (%) = percentage of a donor panel reactive with the patient's serum. †An unacceptable mismatch. ‡Not inherited maternal HLA-A antigen is identical to paternal HLA-A antigen in the patient or mother is homozygous on HLA-A locus.

We do not know whether nonresponsiveness to the noninherited maternal class II antigens also exists. We found no evidence that a DR incompatibility on the not inherited maternal haplotype increases the chance to develop nonresponsiveness toward the maternal class I antigens, as is suggested in neonatal tolerance in the mouse (*12*).

Our findings raise a number of questions both of a fundamental and a practical character. For instance, we do not know whether what holds true for antibody formation also holds true for T cell activation. It is also unknown whether patients who are not highly sensitized can be nonresponders against NIMA; that is, whether sensitization plays a role in activating a (latent) nonresponder status.

The guidelines for selecting organ or bone marrow donors should perhaps be reconsidered. Fifteen of the 26 patients studied showed B cell unresponsiveness against the NIMAs; therefore, about half of the patients mismatched for one (or sometimes two) HLA class I (and maybe also class II antigens), if these are identical to the NIMA, may have graft survival similar to that of HLA identical grafts. Thus a larger percentage of patients could be provided with an organ or a bone marrow transplant with a better than average prognosis.

It may now be insufficient to ask whether a disease, X, is significantly associated with a certain HLA antigen, Z, in the patient. Patients suffering from disease X, and who are HLA-Z negative, should be checked for tolerance to a NIMA. It then still remains to be established whether Z is necessarily the tolerating antigen. Such a hypothesis might be supported by observations that show a significantly decreased frequency of HLA-B5 in the mothers of patients suffering from rheumatic fever (*13*). Also, experiments in the mouse indicate that neonatal tolerance to MHC antigens alters the Ir gene control of the cytotoxic T cell response to vaccinia virus (*14*).

In conclusion, the high frequency of NIMAs among the permissible mismatches of highly sensitized patients suggests that the fetus cannot only immunize its mother (*15, 16*), but that the mother can also induce partial, but lifelong, tolerance in some of her children. Further studies should reveal whether the nonresponsiveness is due to clonal deletion, suppressor cells, or other mechanisms.

REFERENCES AND NOTES

1. R. E. Billingham, L. Brent, P. B. Medawar, *Nature* **172**, 603 (1953).
2. J. W. Streilein and J. Klein, *J. Immunol.* **119**, 2147 (1977).
3. G. F. J. Hendriks *et al.*, *Scand. J. Urol. Nephrol.* **92**, 81 (1985), and unpublished observations.
4. F. H. J. Claas *et al.*, *Transpl. Proc.*, in press.
5. F. H. J. Claas *et al.*, unpublished observations.
6. J. Bodmer and W. Bodmer, *Immunol. Today* **5**, 251 (1984).
7. D. R. Jacoby, L. B. Olding, M. B. A. Oldstone, *Adv. Immunol.* **35**, 157 (1984).
8. X. Chardonnens and M. Jeannet, *Tissue Antigens* **15**, 401 (1980).
9. R. D. Owen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **40**, 420 (1954).
10. J. W. Streilein, R. S. Gruchella, P. Wood, P. Strome, *Ann. N.Y. Acad. Sci.* **392**, 276 (1982).
11. J. W. Streilein and R. S. Gruchella, *Immunogenetics* **12**, 161 (1981).
12. J. W. Streilein, *Immunol. Rev.* **46**, 125 (1979).
13. S. E. Read *et al.*, *Transpl. Proc.* **9**, 543 (1977).
14. A. Müllbacher, R. V. Blanden, M. Brenan, *J. Exp. Med.* **157**, 1324 (1983).
15. J. J. van Rood, J. G. Eernisse, A. van Leeuwen, *Nature* **181**, 1735 (1958).
16. R. Payne and M. R. Rolfs, *J. Clin. Invest.* **37**, 1756 (1958).
17. J. J. van Rood, in *NIAID Manual of Tissue Typing Techniques*, J. G. Ray, D. B. Hare, P. D. Pedersen, D. I. Mullally, Eds. (National Institutes of Health, Bethesda, 1976), p. 81.
18. J. W. Bruning *et al.*, *Human Immunol.* **5**, 225 (1982).

19. Supported by the Dutch Foundation for Medical and Health Research (MEDIGON) and the J. A. Cohen Institute for Radiopathology and Radiation Protection (IRS) (grant no. 3.3.1). The Eurotransplant Foundation gave logistic support and we thank the dialysis centers for their help in contacting the parents of the patients.

29 April 1988; accepted 18 August 1988

Hyperthermia Protects Against Light Damage in the Rat Retina

MARY F. BARBE,* MICHAEL TYTELL, DAVID J. GOWER, WILLIAM J. WELCH

An increase in the synthesis of heat shock proteins that is induced in cells in vitro by hyperthermia or other types of metabolic stress correlates with enhanced cell survival upon further stress. To determine if a similar increase in stress tolerance could be elicited in vivo, rats were made hyperthermic, and then their retinas were tested for sensitivity to light damage. This treatment resulted in a marked decrease in photoreceptor degeneration after exposure to bright light as compared to normothermic animals. Concomitant with such protection was an increase in retinal synthesis of three heat shock proteins. Thus, a physiological rise in body temperature enhances the stress tolerance of nerve tissue, perhaps by increasing heat shock protein production.

MANY ORGANISMS PRODUCE A family of "heat shock" or "stress" proteins (HSPs) after hyperthermia (*1–3*). The production of HSPs correlates with the acquisition of tolerance to a wide variety of stressors in cultured cells and tissues (*2, 4–7*). For example, exposure of cells to a mild heat challenge greatly enhances cell survival after a subsequent, and what would otherwise be a lethal, elevation in temperature (*4–7*). This protective effect, referred to as acquired thermotolerance, is correlated with the expression of the HSPs. In the central nervous system (CNS), the stress response may be of crucial importance to the outcome of injury if it is involved in cell survival. This response is a general feature of CNS reaction to injury, since HSP production is elevated by D-lysergic acid diethylamide (LSD) (*8*), hyperthermia (*9, 10*), ischemia (*11*), sodium arsenite (*12*), surgical cutting (*13*), and concussive injury (*14*). We have begun to explore this response by using the retina as a model for the CNS. Prolonged or excessive exposure of the retina to light results in irreversible damage to the photoreceptor cells (*15–18*). If a mild heat stress in vivo also leads to enhanced stress tolerance, then perhaps prior hyperthermia in the rat could reduce death of photoreceptor cells caused by subsequent exposure to bright light, and thus reduce damage to the retina. We report here that a brief period of hyperthermia before light exposure significantly reduced photoreceptor degeneration while it stimulated HSP production.

We and another group have shown that production of one or more HSPs can be stimulated in the retina after a brief period of heat stress and that the pattern of these newly synthesized retinal HSPs as revealed by two-dimensional gel electrophoresis was very similar to that reported in other systems (*9, 10*). This procedure, combined with a well-established protocol for quantitation of light damage in the rat retina (*15–17, 19*),

M. F. Barbe, Department of Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19144.
M. Tytell, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.
D. J. Gower, Section of Neurosurgery, University of Oklahoma, Oklahoma City, OK 73126.
W. J. Welch, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

*To whom correspondence should be addressed.