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Monoclonal antibodies to DNA modified by 8-methoxypsoralen and ultraviolet A light

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ABSTRACT

A panel of monoclonal antibodies have been developed which specifically recognize DNA modified by 8-methoxypsoralen (8-MOP) and ultraviolet A light (320-400 nm) (UVA). These antibodies have been characterized as to sensitivity and specificity by an enzyme linked immunosorbent assay (ELISA). In a competitive ELISA with the most sensitive antibody, 50% inhibition of antibody binding occurred at 17 fmole 8-MOP-DNA photo adducts. One adduct per 10^7 bases could be reliably detected. There was also some antibody cross-reactivity with DNAs modified by 4′ aminomethyl-4, 5, 8-trimethylpsoralen and 4′, 5-dimethylangelicin as well as DNA isolated from cells treated with 8-MOP and UVA. The primary specificity of one of the antibodies was shown to be the 4′, 5′ thymine monoadduct by competitive inhibition studies using HPLC fractions of an enzymatic digest of 8-MOP poly(dA-dT).poly(dA-dT) . These antibodies should allow the quantitation of adduct levels in various *in vitro* systems as well as humans exposed clinically to 8-MOP and UVA.

INTRODUCTION

8-Methoxypsoralen (8-MOP) plus ultraviolet A light (UVA 320-400 nm) is used clinically in the treatment of psoriasis, a hyperproliferative disease of the epidermis (1). More recently, 8-MOP plus UVA (PUVA) has been used extracorporeally as a cytoreductive treatment for the leukemic phase of cutaneous T cell lymphoma, as well as a form of immunosuppression in the treatment of autoimmune disorders (2). 8-MOP, when photoactivated with UVA light reacts with pyrimidine bases, primarily thymine, in cellular DNA (3). Psoralens are polyfunctional DNA reagents which form three distinct types of photoadducts: two monoaddition products and one diadduct or cross-link. The monoadducts are either 3,4-pyrone side cyclobutyl adducts with the 5,6 bond of thymine or similar cyclobutyl adducts with the 4′, 5′-furan side of 8-MOP. The 3,4 adduct has a weak maximum near 300 nm while the 4′, 5′-adduct has a strong absorption band near 330 nm (4). Thus, the absorption of another UVA photon by a 4′, 5′-furan side monoadduct results in the photoactivation of its 3,4-pyrone

carbon-carbon double bond and subsequent addition to a thymine on the opposite strand to give a cross-linked adduct. HPLC analysis of calf thymus DNA irradiated with UVA in the presence of 8-MOP has shown that the 4′, 5′-monoadduct is the predominant photoadduct (5). In vitro studies of cultured human cells have shown that these photoadducts inhibit DNA synthesis (3), are mutagenic (6) and may also be carcinogenic (7). Psoralen adducts represent fairly bulky additions to DNA and thus serve to activate cellular mechanisms which appear to recognize conformational changes in DNA structure (8).

Recently it has become possible to quantitate specific modifications on DNA by immunological methods. Monoclonal antibodies have been developed which specifically recognize the DNA adducts of several carcinogens including benzo[a]pyrene (9), aflatoxin (10,11) and ethyl nitrosurea (12) as well as thymidine dimers (13) and thymine glycol (14) produced in DNA exposed to ionizing or UV radiation. These antibodies can be used in highly sensitive enzyme linked immunosorbent assays (ELISA) to detect femtomole levels of adducts. With this level of sensitivity, it is possible to quantitate adduct levels in various animal and human samples.

A previous study reported the production of a rabbit polyclonal antibody to 8-MOP-DNA (15) which was used for immunofluorescence studies to detect damaged DNA in slide fixed tissue preparations treated with 8-MOP and UVA. However, the polyclonality of this antiserum did not permit distinction between different types of photo adducts nor was the sensitivity of the assay quantitated in terms of the amount of adduct that could be detected.

Here we report the development of a panel of monoclonal antibodies to 8-MOP-DNA. These antibodies have been characterized as to sensitivity and specificity by ELISA.

MATERIALS AND METHODS

8-Methoxypsoralen (8-MOP) (Sigma Chem. St. Louis, MO.), [^{3}H]8-MOP and 4′aminomethyl-4,5′,8-trimethylpsoralen (AMT) (HRI Associates, Emeryville, CA.) were used without further purification. 4′, 5-Dimethylangelicin (DMA) was a gift from Dr. Rodighiero, Padua. Poly(dA-dT).poly(dA-dT) and poly(dA).poly(dT) were purchased from Pharmacia P.L. Biochemicals, Milwaukee, WI. Calf thymus DNA, goat anti mouse IgG and goat anti rabbit IgG-alkaline phosphatase conjugates, and p-nitrophenyl

phosphate (Sigma 104) were purchased from Sigma. Rabbit anti mouse IgG_1, IgG_2, IgM or kappa chains were purchased from Litton Bionetics, Charleston, SC. The media F12 (HAM's) and Iscove's modified Dulbecco's Medium (IMDM) were purchased from Grand Island Chem. Co., Grand Is. NY. Fetal calf serum (FCS) was purchased from Sterile Systems, Logan, Utah.

Modification of DNA

PBS solutions of calf thymus DNA (1 mg/ml) and 8-MOP (0.1 mg/ml) with a trace of [^{3}H]-8-MOP were irradiated with near UV light (Westinghouse). After irradiation the solutions were exhaustively dialyzed against distilled water to remove unbound 8-MOP. The level of 8-MOP photomodification was determined by liquid scintillometry (Searle Mark II). The synthetic polymers were modified by a similar procedure, as was DNA when modified by AMT or DMA.

Immunization with 8-MOP-DNA

BALB/cCr mice (6-8 weeks old) (West Seneca Laboratories, West Seneca, NY) were treated as follows: Week 1: intraperitoneal (i.p.) injection of 100 µg 8-MOP-DNA 11% modified complexed with an equal amount of methylated bovine serum albumin (mBSA) and emulsified with an equal volume of complete Freunds adjuvant (FA). Week 3: 200µg 8-MOP-DNA-mBSA complex with incomplete FA (i.p.). Week 5: 75 µg 8-MOP-DNA-mBSA complex with incomplete FA (i.p.). Week 7: 50 µg 8-MOP-DNA-mBSA complex without FA, intravenous injection in tail vein.

During week 6, blood samples were removed from the tail and assayed by ELISA for antibody activity, as described below.

Cell fusion

The myeloma cell line P3X63-AG.8.653 was grown in a basal medium (BM) (F12/IMDM+α thioglycerol + progesterone + pen-strep) supplemented with a mixture of trace elements (TE) and 1% FCS (16). The selection media, 10 X HT and HAT, were prepared as described (17).

Cell fusions were carried out essentially as described previously (9). Briefly, mouse spleens were removed three days after the final immunization. Cells were treated with 0.14M Tris (pH 7.2), 0.017M NHCl to remove red blood cells, mixed in a 10:1 ratio with the myeloma cells and pelleted. Cells were fused with 0.5 ml 41.6% PEG (PEG1000, Baker Chem. Co., Philipsburg, NJ)/15% DMSO in BM+TE+10% FCS followed by 0.5 ml 25% PEG in BM+TE+10% FCS (17). The volume was brought to 90 ml with normal media. After 4 hr, 10 ml 10 X HAT was added and 0.1 ml distributed into ten 96 well Corning #25860 (Corning, NY) tissue culture plates. After 6

days, 0.1 ml HT medium was added to each well. The supernatants were screened on day 14, by ELISA as described below.

Cells from positive wells were subcloned in agarose plates containing an underlying monolayer of CREF cells. The CREF cells were first plated at a density of 2.5 X 10^4 cells per 50 mm dish. The next day, 5 ml of 0.50% agarose (Sea Plaque, FMC Corp.) in BM + TE + 10% FCS medium were added on top of this monolayer and the plates incubated at 4^oC for 10 min. Hybridoma cells (10^2 - 10^3) were added in 1 ml 0.50% agarose in BM + TE + 10% FCS medium and the plates were incubated at 4^oC for 10 min. After growth at 37^oC for 5 - 10 days, clones were picked and grown in 96 well plates for about 2 weeks before screening the supernatants by ELISA.

Screening of hybridomas

Polystyrene U-bottom micro plates (Corning #25855) were coated with 20 ng 8-MOP-DNA (0.50% modified) in PBS, by drying at 37^oC overnight. The plates were then washed with PBS-Tween using an automatic plate washer (Flow Multiwash, McLean VA.) set for five 200 µl washes. A similar wash step was done after each incubation. Non-specific binding to the plate was then minimized by incubating the wells with 200 µl 1% FCS in PBS-Tween for 1 hr. The hybridoma supernatants were then transferred from the storage plates into individual wells of the coated plates using a replica transfer technique (9). After a 1.5 hr incubation 37 ^oC , the supernatants were flipped back into the storage plates. Goat anti mouse IgG-alkaline phosphatase (diluted 1:1000, 0.1 ml) was then added to each well and incubated for 1.5 hr. After washing the plates with PBS-Tween, 0.1 ml p-nitrophenyl phosphate in 1 M diethanolamine (pH 8.6) was added to each well. The plates were incubated for 60 min and the color development at 405 nm was read on a Flow Multiscan MC recorder. Positive cultures were then rescreened by adding 50 µl of their supernatants to each of three wells, one coated with 8-MOP-I-DNA, one coated with unmodified DNA and one blank, i.e. a well containing no DNA. This process eliminated those clones producing antibodies to unmodified DNA or to some unknown antigen present in the uncoated plate.

Noncompetitive and competitive ELISA assay

Polystyrene 96 well plates (Immulon 2, Dynatec Lab., Alexandria Va.) were coated with varying amounts of 8-MOP modified and unmodified DNA, as described above for the competitive assay. Dilutions of the hybridoma supernatants were then added, and further steps carried out as described above.

For the competitive assay, wells were coated with 5 ng 8-MOP-I-DNA (0.50% modified). The hybridoma supernatant was diluted and mixed with an equal volume of the competitor before adding 0.1 ml of this mixture to the well. Further steps were performed as described in the non-competitive assay. For additional details, see figure legends.

Isotype determination

Hybridoma supernatant (0.1 ml of 1:500 dilution) was added to wells coated with 5 ng 8-MOP-I-DNA. After incubation, rabbit antiserum specific for mouse IgG_1, IgG_2, IgM or kappa chains (1:500 dilution) were added for 1.5 hr. This step was followed by the addition of goat anti rabbit IgG-alkaline phosphatase (1:500) for 1.5 hr. The substrate was then added and the enzyme activity determined as described above.

Treatment of myeloma cells with 8-MOP

The myeloma cells were centrifuged, washed, and resuspended in 10 ml of PBS containing 500 ng/ml 8-MOP. Cells were irradiated with near ultraviolet light (Westinghouse FT#) with an irradiance of 4, 5 mW/cm^2 for 60 minutes (16 J/cm^2 incident dose). 8-MOP modified DNA was isolated as described previously (18).

Enzymatic hydrolysis and HPLC analysis

8-MOP modified deoxypolynucleotides were enzymatically digested to nucleosides as described previously (5). The hydrolysate (200 µl) was applied to an octadecylsilane (ODS) reversed phase column (Altex) and eluted using a methanol 20m M-potassium phosphate buffer (pH 3.5) gradient. The fractions were lyophilized, the pH adjusted to 7, and an aliquot used in a competitive inhibition assay. One ml fractions from the HPLC of another 200 µl aliquot of the same sample were collected and analyzed by liquid scintillation. Each sample was counted for 10 minutes.

RESULTS

BALB/cCr mice were immunized with calf thymus DNA modified to an extent of 5.5% with 8-MOP and electrostatically complexed to methylated bovine serum albumin. Sera from the animals were tested six weeks after initial injection and screened for specific antibody by ELISA, as described above. Spleen cells from a positive mouse were fused with mouse myeloma cells (P3X63-AG.8.653) and cultured in HAT selection medium in 1000 micro wells. Two weeks after fusion, supernatants were screened by ELISA for antibody specific for 8-MOP-DNA. Positive clones were sub-

Table I. Isotype analysis of Monoclonal Antibodies

	Absorbance at 405 nm			
	3A8	5G3	8G1	9D8
IgG_1	.618	.061	.117	.080
IgG_2	.070	.089	1.027	1.299
IgM^2	.016	1.670	.098	.060
Kappa	.420	1.225	.526	.502

Hybridoma supernatants were tested at a 1:500 dilution on a 5 ng 8-MOP-DNA coated plate. Rabbit antisera specific for mouse IgG_1, IgG_2, IgM, or kappa chain (1:500 dilution) were used in the second incubation stage before adding goat anti-rabbit IgG-alkaline phosphatase conjugate (1:500 dilution). For additional details, see Materials and Methods.

cloned in agarose and retested for antibody production. A total of 14 stable clones specific for 8-MOP-DNA were produced. Detailed characterization of four of these clones are described here.

The isotype classification of the antibodies were determined by ELISA using rabbit antiserum specific for mouse IgG_1, IgG_2, IgM, and kappa chains (Table I). Antibody 3A8 is IgG_1, kappa, antibody 5G3 is IgM, kappa, and antibody 8G1 and 9D8 are IgG_2 kappa.

The specificity of the antibodies was tested by noncompetitive ELISA. Microplates were coated with increasing amounts of 0.50% modified 8-MOP or nonmodified DNA. After incubation with antibody 8G1, the plates were washed and incubated with goat anti mouse IgG alkaline phosphatase followed by p-nitrophenylphosphate. The absorbance at 405 nm, a measure of antibody binding, increased when increasing amounts of 8-MOP modified DNA was coated on the plate (Fig. 1). There was no cross-reactivity with nonmodified DNA. The lowest amount of modified DNA that could be differentiated from nonmodified DNA (greater than 5-fold difference in absorbance of modified over nonmodified) is 0.2 ng. This corresponds to 3 fmole of bound psoralen.

Competitive ELISA was used to determine the sensitivity of the antibodies and to obtain additional information about their specificity. Plates were coated with 5 ng of 0.50% modified 8-MOP-DNA and the antibody binding to the plate competed off by the addition of increasing amounts of 8-MOP-DNA in solution. Antibody 8G1 binding to the plate is inhibited 50% by 17 fmole of 8-MOP adduct/well (Fig. 2). This corresponds to about 1 ng of 0.50% modified DNA. The addition of up to 50 µg of nonmodified

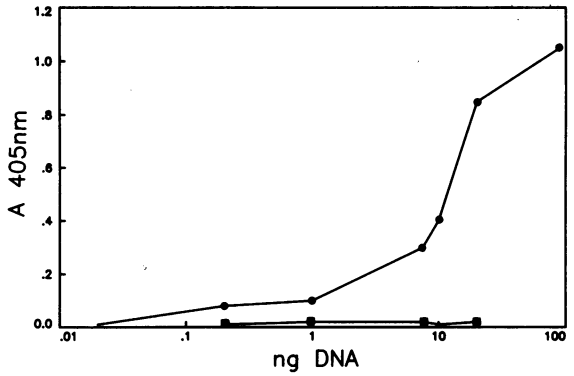

Figure 1. Non-competitive binding of monoclonal antibody 8G1 to increasing amounts of DNA. The indicated amount of unmodified calf thymus DNA (■) or 0.50% modified 8-MOP-DNA (●) were absorbed to each well. The antibody was used at a 1:500 dilution.

DNA per well does not inhibit antibody binding to the plate (data not shown). Additional information about antibody specificity was obtained by testing 8G1 with several synthetic polymers. The data for 7.0% modified 8-MOP-poly(dA-dT).poly(dA-dT) and 1.3% modified 8-MOP-poly(dA)-.poly(dT) are also given in Figure 2. The 50% inhibition values were 77 fmole and 13 fmole, respectively. Another sample of poly(dA-dT)-.poly(dA-dT) modified to a level of only 0.49% had a 50% inhibition value

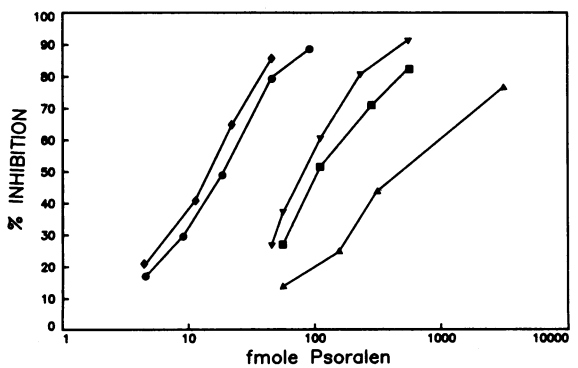

Figure 2. Competitive inhibition of monoclonal antibody 8G1 binding to 8-MOP-DNA. The competitors were 0.50% modified 8-MOP-DNA (●), 7.0% modified 8-MOP-poly(dA-dT).poly(dA-dT) (▼), 1.3% modified 8-MOP-poly(dA)-.poly(dT) (◆), 3.4% modified DMA-DNA (■) and 1.9% modified AMT-DNA (▲). The antibody was used at a 1:60,000 dilution.

Table II. Competitive Inhibition of Antibody Binding fmole Causing 50% Inhibition

	Modification Level	3A8	5G3	8G1	9D8
8-MOP-DNA	0.50%	67	32	17	51
8-MOP-poly(dA-dT)	7.0%	595	83	77	98
8-MOP-poly(dA).poly(dT)	1.3%	84	43	13	13
DMA-DNA	3.4%	260	155	104	100
AMT-DNA	1.9%	230	58	580	870

at 75 fmole. The cross-reactivity of the antibody with two other psoralens, 4-aminomethyl-4,5′, 8-trimethylpsoralen (AMT) and 4′, 5-dimethylangelicin (DMA) was also tested. Antibody 8G1 reacted better with 3.4% modified DMA-DNA (50% inhibition at 104 fmole) than with 1.9% modified AMT-DNA (50% inhibition at 580 fmole). Antibody 8G1 was also tested against free 8-MOP. Up to 1.5 x 10^5 fmole per well did not inhibit antibody binding. Two other carcinogen modified DNAs were also tested. Neither benzo[a]pyrene diol epoxide modified DNA (BPDE-DNA) or acetylaminofluorene modified DNA showed any cross-reactivity with antibody 8G1 when tested at levels of 10^5 fmole per well. The 50% inhibition values for the various competitors for antibody 8G1 and the other three clones are summarized in Table II. With the exception of 8G1, all the antibodies show their highest reactivity with the original immunogen 8-MOP-DNA. The reactivity for the psoralen adduct is similar whether it is on DNA or poly(dA).poly(dT) but reactivity is 2 to 30-fold lower with AMT or DMA modified DNA.

To further characterize antibody specificity, [^{3}H]8-MOP modified poly(dA-dT).poly(dA-dT) was enzymatically digested and chromatographed by HPLC. Figure 3a is the UV profile and 3b is the radioactive trace. A major UV and radioactive peak occurs at fractions 28-29. This peak has been identified previously as the 4′,5′ monoadduct (5). Another major UV peak occurs at fraction 19 and has been identified as thymine. No radioactivity occurs at this position. A major radioactive peak occurs at fraction 6 and may be due to tritium exchange or undigested oligonucleotides. In addition, there are a number of small UV and radioactive peaks probably due to minor monoadducts and various cross-linked adducts. Aliquots of the fractions were also evaporated, neutralized and tested for reactivity with antibody 8G1. Figure 3c indicates strong antibody reactivity with the 4′,5′ monoadduct peak. There is also considerable antibody reactivity with the minor unidentified radioactive peaks.

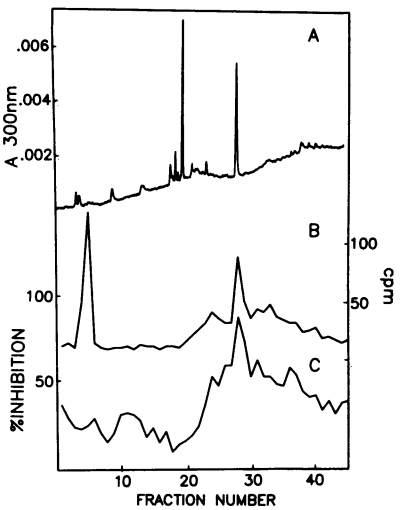

Figure 3. HPLC profile of deoxynucleoside adducts from enzymatic hydrolysates of 8-MOP modified poly(dA-dT).poly(dA-dT). (A) UV trace, absorbance at 300 nm, on left axis. (B)[^{3}H] cpm of fractions on right axis. (C) % Inhibition of antibody 8G1 binding to 8-MOP modified DNA for each fraction. Methanol-buffer fractions were evaporated and neutralized before incubation with antibody. The antibody was used at a 1:60,000 dilution.

The cross-reactivity of antibody 8G1 with DNA isolated from cultured cells that had been treated with 8-MOP and UVA light was also investigated. Figure 4 shows that the antibody does cross-react with DNA isolated from treated cells but not with DNA isolated from untreated controls. Based on the 50% inhibition value of 17 fmole for *in vitro* modified DNA, the modification level is calculated to be 3.2 adducts per 10^6 nucleotides.

DISCUSSION

A panel of monoclonal antibodies that recognize 8-MOP modified DNA have been generated and characterized. These antibodies were shown to be

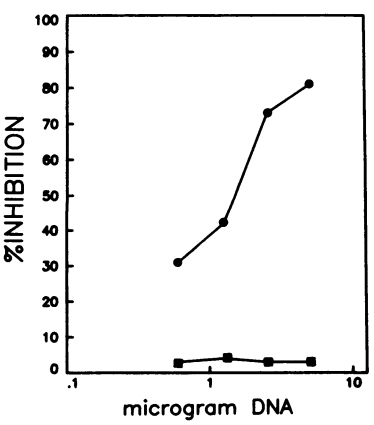

Figure 4. Competitive inhibition of monoclonal antibody 8G1 binding to 8-MOP DNA. The competitors were myeloma DNA obtained from cells exposed to 500 ng/ml 8-MOP and UVA as described in Materials and Methods, (●) and DNA from unexposed controls (■). The antibody was used at a 1:60,000 dilution.

highly specific for modified DNA and showed no cross-reactivity with non-modified DNA. No significant difference was seen in the 50% inhibition values when denatured modified DNA was compared to native modified DNA (not shown). This is in contrast to the results seen with a monoclonal antibody to BPDE-DNA in which denatured DNA reacted almost two orders of magnitude better than native DNA (9). This was attributed to the adduct being more accessible to the antibody when on single stranded DNA. It may, however, be due to the fact that the original immunogen for the BPDE-DNA antibodies was denatured while for the 8-MOP-DNA antibodies it was native. Another possibility is that cross-links on 8-MOP-DNA may cause efficient reannealing after heat denaturation. The polyfunctional nature of psoralens complicates the determination of the specificities of the monoclonal antibodies. Thus, two synthetic polynucleotides, poly(dA).poly(dT) and poly(dA-dT).poly(dA-dT) were modified with 8-MOP. In poly(dA).poly(dT), although the double helical structure enhances 8-MOP modification, only monoadducts form because thymines do not occur on opposite strands. Poly(dA-dT).poly(dA-dT) contains both monoadducts and a small fraction of cross-links. The 4′,5′ furan side monoadduct is predominant.

The data for 8G1 in Table II indicates that poly(dA).poly(dT) showed the greatest activity as a competitive inhibitor of antibody binding. This result suggests that 8G1 recognizes 8-MOP monoadduct regions. The six-fold lower sensitivity toward the monoadduct-rich sample of poly(dA-dT).poly(dA-dT) was unexpected. There was the possibility that the higher modification level of poly(dA-dT).poly(dA-dT) (7.0%) compared to poly(dA).poly(dT) (1.3%) resulted in some lowered cross-reactivity because of clustering adducts. This was ruled out since a sample of poly(dA-dT).poly(dA-dT) with only 0.49% modification had a similar 50% inhibition value (75 fmole) as that with 7.0% (77 fmole). An alternate explanation may be that the unusual backbone conformation of poly(dA-dT)-.poly(dA-dT) (19) may go unrecognized by monoclonal antibodies elicited in response to 8-MOP-modified calf thymus DNA. Additional information about antibody specificity is obtained from the HPLC analysis of modified poly(dA-dT).poly(dA-dT). The highest antibody reactivity was with those fractions containing the 4′,5′ monoadduct.

All the antibodies have some cross-reactivity with AMT and DMA modified DNA. Since 8-MOP and AMT differ only in the methyl and methoxy substitution around the ring, cross-reactivity would be expected. Angeli-

cin, however, differs in its ring structure being an angular psoralen derivative capable of forming only monoadducts (3). Its reactivity indicates that the antibodies recognize structures that are present on the coumarin ring. While the antibody may cross-react with cross-linked adducts, it probably does not specifically recognize the cyclobutyl moieties.

Antibody 8G1 also reacts with DNA isolated from cells treated in culture with 8-MOP and UVA. It was important to demonstrate cross-reactivity with this DNA as well as DNAs treated in vitro if the antibodies are to be used to quantitate adduct levels in patients exposed to psoralen. With the sensitivity of antibody at 17 fmole bound psoralen and the ability to assay 50 µg of DNA in a micro well, it is possible to quantitate one adduct in 10^7 to 10^8 bases. This level of sensitivity should make these antibodies extremely useful in quantitating adducts in various in vitro systems, as well as in humans exposed to psoralen. In addition, these results indicate additional information can be obtained about antibody specificity by combining the immunoassays with analytical HPLC separation techniques.

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REFERENCES

1. Parrish, J.A., Fitzpatrick, T.B., Pathak, M.A., and Tannenbaum, L. (1974), NE J. Medicine 291, 1207-1211.
2. Edelson, R.L., Berger, C.L., Gasparro, F.P., Taylor, J.A., and Lee, K. (1983), Clin. Res. 31, 467A.
3. Song, P.S., and Tapley, J.K. (1979), Photobiol. 29, 1177-1197.
4. Dall Acqua, F.S., Marciani Magno, S., Zambon, F., and Rodishiero, G. (1979), Photochem. Photobiol. 29, 489-495.
5. Gasparro, F.P., Bagel, J., and Edelson, R.L. (1984), Photochem. Photobiol. In press.
6. Sinden, R.R., and Cole, R.S. (1978), J. Bacteriol. 136, 538-547.
7. Stern, R.S., Laird, N., Melski, J., Parrish, J.A., Fitzpatrick, T. B., and Bleich, H.L. (1984), NE Nsl. J. Med. 310, 1156-1161.
8. Hanawalt, P.C., Cooper, P.K., Ganesan, A.K., and Smith, C.A. (1979), Ann. Rev. Biochem. 48, 783-836.
9. Santella, R.M., Lin, C.D., Cleveland, W.L., and Weinstein, I.B. (1984), Carcinogenesis 5, 373-377.
10. Haugen, A., Groopman, J.D., Hau, I.C., Goodrich, G.R., Wogan, G. W., and Harris, C.C. (1981), Proc. Natl. Acad. Sci. U.S.A. 78, 4124-4127.

11. Hertzog, P.J., Smith, J.R.L., and Garner, R.C. (1982), Carcinogenesis 3, 825-828.
12. Rajewsky, M.F., Muller, R., Adamkiewicz, J., and Drosdziok, W. (1980), Carcinogenesis: Fundamental Mechanisms and Environmental Effects. Dordrecht, Holland: Reidel Press, pp. 207-218.
13. Strickland, P.T., and Boyle, J.M. (1981), Photochem. Photobiol. 34, 595-601.
14. Leadon, S.A., and Hanawalt, P.C. (1983), Mut. Res. 112, 191-200.
15. Zarebska, Z., Jarzabek-Chorzelska, R., Zesa, G., Glinski, W., Pawinskia, M., Choozelski, T., and Jablonska, S. (1984), Photochem. Photobiol. 39, 307-312.
16. Cleveland, W.L., Wood, I., and Erlanger, B.F. (1981), Jr. of Immunol. Meth. 56, 221-234.
17. Pearson, T.W., Pinder, M., Rodants, G.E., Kor, S.K., Ludin, L.B., Mayor-Withay, K.S., and Hewett, R.S. (1980), Jr. of Immuno. Meth. 34, 141-154.
18. Brown, H.S., Jeffrey, A.M., and Weinstein, I.B. (1979), Cancer Res. 39, 1673-1677.
19. Shindo, H., Simpson, R.T., and Cohen, J.S. (1979), J. Biol. Chem. 254, 8125-8129.