Hydrogen peroxide induced adenosine diphosphate ribosyl transferase (ADPRT) response in patients with inflammatory bowel disease

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SUMMARY The sample population in this initial case control study of the adenosine diphosphate ribosyl transferase (ADPRT) response of inflammatory bowel disease patients included: 23 patients with ulcerative colitis (UC) – active and inactive, 13 patients with Crohn's disease (CD) – active and inactive, 14 first degree relatives of UC and CD patients, and 19 age-matched controls. Adenosine diphosphate ribosyl transferase activity was determined after one hour incubation with 1% plasma (the constitutive value) or with 1% plasma and 100 μM H_2O_2 (the activated value) with the resulting difference designated as the induced value. Statistically significant decrease in ADPRT activity was found for the constitutive, activated and induced values in human mononuclear leucocytes of UC and CD patients, compared with controls. The values in the first degree relatives of UC and CD patients were not significantly different from either the control or disease populations, indicating an intermediate ADPRT response. These results may be related to the nature of the immunological response of IBD patients and comparable with similar findings in other diseases with known DNA repair deficiencies – for example, colon cancer.

Ulcerative colitis (UC) and Crohn's disease (CD) are both inflammatory bowel diseases (IBD) whose origins remain obscure.¹⁻⁷ Although they are usually differentiated by their clinical manifestations, these diseases have a number of common features; these include an incidence of colorectal cancer greater than that found in the general population^{3,8-10} and an increased familial incidence of IBD.¹⁻⁴ In addition, immunological perturbations occur during the active state of the disease^{1-7,11-15} and although they may not be the initiating factors, they are associated with the continued chronic inflammation found in both UC and CD patients.

Our interest focused on the DNA repair response of IBD patients because the above features paralleled those found in our previous studies. These showed that a reduced level of DNA repair, as measured by the rate of unscheduled DNA synthesis

(UDS) and adenosine diphosphate ribosyl transferase (ADPRT) induction, occurred in patients with a history of colorectal cancer as well as in individuals genetically predisposed to colorectal cancer and in patients with colonic adenomatous polyps.¹⁶⁻¹⁹ Other diseases which are known to be genetically predisposed to DNA repair deficiencies such as xeroderma pigmentosum and ataxia telangiectasia²⁰ also show a significant cancer predisposition.

The present study was initiated to determine the DNA repair capacity of IBD patients as measured by the inducibility of the repair related enzyme, ADPRT. This enzyme transfers the adenosine diphosphate moiety of NAD^+ to various acceptor proteins in the form of $[\text{ADP-ribose}]_n$ monomers and polymers. Although the precise function of the ADPRT enzyme is not completely understood, it has been shown to function during the process of DNA excision repair after the induction of DNA damage²¹⁻²³ and also during the process of cellular differentiation,²⁴ including the response of human lymphocytes to mitogenic stimulation.²⁵

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Hydroperoxides and other reactive oxygen species are known to be involved in the processes of mutagenesis, aging and tumour promotion²⁶⁻²⁸ and, in addition, have also been shown to elicit DNA repair responses.²⁹ Creating an environment of oxidative stress – that is, the exposure of cells to oxygen radicals – may well parallel the condition found in IBD patients whose intestinal mucosa contains increased numbers of monocytes and macrophages which are known to kill invading bacteria by sudden oxidative bursts.³⁰

As related below, a statistically significant reduction in ADPRT was found in HML of IBD patients after incubation with either 1% plasma or 100 μ M H₂O₂ in 1% plasma.

Methods

PATIENTS

The patients included in this investigation were all selected for their past or present history of inflammatory bowel disease. Others included in this study were first degree, clinically disease free, relatives of IBD patients and a control population of disease free individuals with no family history of IBD. Sampling of patients occurred between April and June, 1987 at the Tel Aviv Medical Center, Israel. At the initial time of sampling patient category was unknown. These individuals were separated into four categories: (1) Controls – those without IBD and no familial history of the disease, n=19; (2) Patients with ulcerative colitis (active and inactive), n=23; (3) Patients with Crohn's disease (active and inactive), n=13; (4) First degree relatives of ulcerative colitis (n=9) and Crohn's disease (n=5) patients (total n=14).

SAMPLING PROCEDURES

The isolation technique for HML has been documented elsewhere.³¹ Samples of peripheral venous blood (~30 ml) were collected in heparinised Vacutainerstm, centrifuged at 100×g for 10 minutes, and the platelet rich plasma, upper layer, was removed. Sterile physiologic saline solution was added to the lower cellular layer to restore it to the original blood volume. The sample was then layered onto Ficoll-Isopaque solution and centrifuged at 400×g for 25 minutes. Mononuclear leucocytes were isolated from the upper phase at the interface, washed with physiologic saline and quantified in a haemocytometer. Plasma was centrifuged at 400×g for 25 minutes to deplete its platelet content.

TREATMENT OF HML WITH H₂O₂

Human mononuclear leucocytes (0.5×10⁶/ADPRT assay) were suspended in physiologic saline supple-

mented with 1% platelet depleted autologous plasma in a total volume of 550 μ l. H₂O₂, dissolved in saline, was added to give a final concentration of 100 μ M. The resulting mixture was incubated for one hour at 37°C. Cells were then pelleted by centrifuging before the determination of the activated ADPRT value. Another set of samples without H₂O₂ was kept under the same culture conditions to determine the background ADPRT value which is designated as the constitutive value. The resulting difference between these two values is termed the induced value.

ADPRT ASSAY

We have published our procedure in detail elsewhere.³² Briefly, duplicate cultures of 0.5×10⁶ cells were pelleted at 4°C and permeabilised in 1.5 ml hypotonic buffer solution (10 mM Tris-HCl, 1 mM EDTA, 30 mM 2-mercaptoethanol, 4 mM MgCl₂, pH 7.8). Cells were then centrifuged and the permeabilisation buffer removed. Adenosine diphosphate ribosyl transferase was estimated after 15 minutes at 30°C in a reaction mixture containing 0.7

Table 1 Control group (n=19). ADPRT values (cpm/0.5×10⁶ cells) of HML with and without treatment with H₂O₂ (100 μ M) for one hour at 37°C in the presence of 1% platelet-poor plasma

Patient code	Age Sex	Constitutive ADPRT	Activated ADPRT	Induced ADPRT
16	40 M	901	2576	1675
20	45 M	698	4023	3325
21	27 M	955	2879	1924
22	34 F	1224	6195	4971
23	46 F	706	3111	2405
24	36 M	1039	2934	1895
25	55 M	1526	6346	4820
32	32 F	993	4017	3024
38	45 M	623	3944	3321
45	38 F	588	4146	3558
46	30 M	539	3482	2943
47	71 M	708	3001	2393
49	42 M	691	4691	4000
68	37 F	494	2548	2054
69	47 F	928	3342	2414
70	34 M	681	2352	1671
76	53 M	626	2365	1739
77	36 M	394	2813	2419
78	34 M	506	3870	3364
Mean (SE) (n=19)	41.2 (2.4)	780 (64.4)	3612 (264)	2838 (230)
Student's <i>t</i> test v all UC and CD patients (n=36)	42 (3.0) p>0.5	598.6 (32) p=0.006	2652.4 (190) p=0.004	2053.2 (174) p=0.01
Student's <i>t</i> test v first degree relatives of UC and CD patients (n=14)	48.9 (4.3) p>0.09	776 (103) p=0.44	2928 (204) p=0.06	2152 (184) p=0.03

Table 2a Ulcerative colitis group (n=23). ADPRT values (cpm/0.5×10⁶ cells) of HML with and without treatment with H₂O₂ (100 μM) for one hour at 37°C in the presence of platelet depleted plasma

Patient code	Age	Sex	Age of onset	Active dis	Constitutive ADPRT	Activated ADPRT	Induced ADPRT
3	58	F	39	—	893	1814	921
5	66	M	31	—	497	1872	1375
8	66	F	62	—	397	1048	651
26	67	M	63	—	781	3445	2664
27	36	M	31	+	753	2968	2215
28	34	F	25	+	855	4810	3955
31	54	M	32	—	869	2859	1990
33	31	M	31	+	830	3976	3146
35	33	M	28	—	601	2443	1842
39	57	M	43	—	615	3835	3220
40	26	F	10	+	760	2285	1525
42	54	M	?	+	597	2476	1879
43	39	M	26	—	542	4330	3788
52	37	M	26	—	546	1904	1358
56	39	F	19	+	753	1104	351
57	77	M	75	+	339	1946	1607
58	61	M	35	—	671	3096	2425
60	25	M	24	+	521	2719	2198
62	25	M	16	—	644	3309	2665
64	65	F	57	+	376	3111	2734
67	33	F	29	+	608	3780	3172
74	19	F	18	—	657	3325	2668
79	63	F	42	—	502	3050	2548
Mean (SE) (n=23)	46.3 (3.6)		34.6 (3.5)		635 (33)	2848 (202.7)	2212 (196)
Student's <i>t</i> test v controls (n=19)	41 (2.4)				780 (64.4)	3612 (264)	2838 (230)
	p>0.3				p<0.04	p<0.02	p<0.04

μCi [Adenosine 2,8-³H]-NAD⁺ (27.1 Ci/mmol). The data were recorded as trichloroacetic acid precipitable cpm per 0.5×10⁶ cells after collection onto nitrocellulose filters. Radioactivity was quantified by scintillation counting over a 10 minute period. The degree of quenching was low and consistent between experimental groups. Measurements were made in duplicate and the paired values were within 10% of

the mean.

STATISTICAL ANALYSIS

Student's *t* test was used for the data analysis found in Tables 1–3. Data analysis in Table 4 was carried out on a Data General MV2000 minicomputer with a software package (version 5) from the SAS Institute, Cary, NC.

Table 2b Crohn's disease group (n=13)

Patient code	Age	Sex	Age of onset	Active dis	Constitutive ADPRT	Activated ADPRT	Induced ADPRT
13	59	M	40	—	510	2611	2101
17	37	M	22	+	594	4054	3460
18	43	F	40	+	838	3320	2482
19	69	F	69	—	723	2260	1537
29	40	F	20	—	966	3739	2773
44	24	F	20	—	356	986	630
50	19	M	17	—	294	443	149
51	40	F	30	—	499	1738	1239
53	20	M	18	+	673	3465	2792
61	24	F	23	—	197	362	165
66	26	M	23	+	493	1271	778
75	48	F	18	—	572	4193	3621
80	50	F	34	+	227	1518	1291
Mean (SE) (n=13)	38.4 (4.3)		28.8 (4.0)		534 (64)	2305 (376.3)	1771 (330)
Student's <i>t</i> test v controls (n=19)	41 (2.4)				780 (64.4)	3612 (264)	2838 (230)
	p>0.5				p<0.01	p=0.006	p=0.01

Table 3 First degree relatives of UC and CD patients (n=14). ADPRT values (cpm/0.5×10⁶ cells) of HML with and without treatment with H₂O₂ (100 μM) for one hour at 37°C in the presence of 1% platelet-depleted plasma

Patient code	Age	Sex	Relative	Constitutive ADPRT	Activated ADPRT	Induced ADPRT
30	37	F	UC	769	2504	1735
34	72	M	UC	670	3445	2775
36	45	M	CD	906	3086	2180
37	63	F	UC	416	1201	785
41	51	M	UC	791	3201	2410
48	29	M	CD	760	2919	2159
54	33	M	UC	614	1892	1278
55	75	M	UC	691	3171	2480
59	38	M	UC	695	2841	2146
63	70	F	CD	457	2162	1705
65	62	F	CD	529	3592	3063
71	35	F	CD	586	3984	3398
72	36	M	UC	978	3292	2314
73	39	M	UC	2000	3704	1704
Mean (SE) (n=14)	48.9 (4.3)			776 (103)	2928 (204)	2152 (184)
Student's <i>t</i> test v UC patients (n=23)	46.3 (3.6)			635 (33)	2848 (202.7)	2212 (196)
	p>0.5			p=0.13	p=0.69	p=0.67
Student's <i>t</i> test v CD patients (n=13)	38.4 (4.3)			534 (64)	2305 (376.3)	1771 (330)
	p>0.1			p=0.06	p=0.15	p=0.31

Results

The IBD patients in this study showed a broad range regarding the age of onset and the duration of their disease. Controls were age matched for the upper and lower limits of the disease population. No statistically significant difference with regard to age was found between the controls and any of the other patient categories (Tables 1–4).

The results of H₂O₂ influenced ADPRT in HML are shown in Tables 1–3. When control individuals (Table 1) are compared by Student's *t* test to the UC population (Table 2a), a statistically significant difference is shown for their constitutive activated ADPRT and induced values; these being *p*=0.04, 0.02, and 0.04, respectively (Table 2a).

An even greater level of significance is found when controls are compared with Crohn's disease patients (Table 2b) where the constitutive, activated ADPRT and induced values are *p*=0.01, 0.006, and 0.01, respectively (Table 2b). In addition, significant values are also obtained when the entire IBD population (Tables 2a, b) is matched against the controls; *p*=0.006, 0.004, and 0.01 (Table 1).

On the other hand, first degree relatives of UC and CD patients do not show the same degree of divergence from the control values as those individuals manifesting the disease (Table 1). Their ADPRT measurements vary between being not statistically significant, constitutive value – *p*=0.44; significant, induced value – *p*=0.3; and of borderline significance, activated value – *p*=0.06. They, therefore, do not approach the degree of difference found when either disease group is compared with the control

population. Furthermore, no level of significance was obtained for either disease group when compared to the first-degree relative population (Table 3). They appear, therefore, as a group whose level of ADPRT induction lies between the control and disease populations. This may well indicate a population enriched for suppressed ADPRT responses.

It was also determined that there was no statistical difference between the values found for patients with active IBD *versus* those whose disease was in remission.

Finally, when the influences of the variables disease, age, sex, and family history were analysed by multivariate analysis of variance, it was found that the only significant influence on variance was the presence or absence of disease (*p*=0.01) (Table 4).

Discussion

Non-specific inflammatory bowel disease consisting of ulcerative colitis and Crohn's disease is most commonly encountered in Northwestern Europe and

Table 4 Multivariate analysis of UC and CD groups v control group

Category	F value	p Value
Disease	6.94	0.01
Age	0.03	0.86
Sex	0.08	0.78
Family HX	1.66	0.20

The presence of disease is the only significant variable in relation to the induction of ADPRT.

North America³³⁻³⁶ and appears to be extremely prevalent among members of the Jewish populations of these regions.^{4,34,37} In the United States, IBD (especially CD) is found to be more common among Jewish people⁴ and a study in Malmö, Sweden found the incidence of CD to be five times greater for Jews than for non-Jews.³⁸

The present study was designed to investigate a Jewish population of IBD patients in Tel Aviv, Israel. These patients were of interest as they showed both active and inactive forms of IBD and because the clinical course of their disease had been well documented – for example, periods of remission and age of onset (Tables 2 and 3).

The results as shown in Tables 1–4 clearly show that IBD patients, both UC and CD, have a marked reduction in ADPRT activity. Furthermore, the decrease in the ADPRT activity of the first degree relative population in comparison with the controls may indicate that this population is also affected by similar immunological perturbations, although to a lesser degree, than their UC and CD relatives. These data parallel our findings of DNA repair deficiencies in colon cancer patients, individuals predisposed to colon cancer and in patients with adenomatous polyps.^{16,17,19} Inflammatory bowel disease patients are also similar to these individuals in that they show familial and genetic patterns of disease inheritance and have a higher than normal frequency of colorectal cancer.^{8-10,39-43}

In addition, IBD is associated with a variety of poorly defined immunological abnormalities which because of their nature and severity may have a direct affect on the degree of ADPRT activity. These immunological changes alter the gastrointestinal tract – for example, make it a more pro-oxidative environment, and may be responsible for initiating the long term, chronic phases of the disease.¹² Changes in the cellular environment may also be responsible for the altered ADPRT levels found in IBD patients.

Various types of cells derived from the immune system are prominent in the intestinal mucosa of IBD patients.^{1,5,30,44} These include T cells, activated T killer cells, macrophages, monocytes, polymorphonuclear leucocytes, basophils, and eosinophils.⁵ All of these cell types may participate in the production of tissue damage and inflammatory gastrointestinal reactions.

Furthermore, Nielson *et al*⁴⁵ have shown that there is an abnormality in arachidonic acid metabolism in patients with chronic IBD. Activated neutrophils were shown to have an increased production of LTB₄ which is considered to be the major pro-inflammatory metabolite of arachidonic acid metabolism by polymorphonuclear leucocytes. The increased production of LTB₄ and other lip-

oxygenase products of arachidonic acid may, therefore, support the constant climate of inflammation and tissue degradation that is found in patients with IBD.^{45,46}

This hypothesis is further supported by the recent work of Nishida *et al* who showed that the colonic mucosa of patients with active UC demonstrates a statistically significant increase in arachidonic acid composition.⁴⁷ These findings coincide with the observed increase in the products of arachidonic acid metabolism – for example, leucotriene B₄ and 5-hydroxyeicosatetra-enoic acid (5-HETE) – found in patients with active UC.^{47,48}

Inflammatory bowel disease patients also show a greater production and release of acid hydrolases in their peripheral blood monocytes *in vitro* as compared with controls.³⁰ Under the influence of immunological stimuli, monocytes are thought to be releasing lysosomal enzymes, thereby perpetuating the intestinal inflammation.⁶

Finally, protease effects on ADPRT have also been reported and may contribute to the molecular mechanism behind IBD.⁴⁹ The inhibition of protease and ADPRT synthesis is known to be associated with a reduction in malignant transformation. In addition, both proteases and ADPRT can be inhibited by similar compounds – for example, benzamide – which suggests that proteases may be involved in the formation and action of poly(ADP-ribose).⁴⁹

In conclusion, we have shown a deficient ADPRT response in the HML of IBD patients and as ADPRT activation relates directly to immune responsiveness,^{25,50} this study may afford an approach to the molecular mechanism behind the initiation of IBD.

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