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Short Communication

CELLULAR IMMUNE FUNCTION STUDY IN AN OVARIAN CANCER-PRONE KINDRED

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A KINDRED with propensity to develop ovarian and other types of cancer has been reported by one of us (H.T.L.) (Lynch *et al.*, 1981). The inordinately high frequency of individuals with low serum IgA levels (Schuelke *et al.*, 1982) in this kindred is consistent with the numerous observations of altered immune functions associated with ovarian cancer in the general population (Badger *et al.*, 1981; Daunter *et al.*, 1979; Gerber *et al.*, 1977; Hess *et al.*, 1979; Hughes 1971; Humphrey *et al.*, 1977; Mandell *et al.*, 1979; Mantovani *et al.*, 1980*a*, *b*; Mikulski *et al.*, 1977; Pattillo *et al.*, 1979*a*, *b*; Poulton *et al.*, 1978; Ueda *et al.*, 1978, 1979). We here report the results of tests relative to cellular immune functions in this kindred.

Informed consent was obtained before blood was taken from kindred members and control population donors who resided near this midwestern kindred. Lymphocytes were isolated and stimulated with mitogens in microculture as previously reported (Chaperon, 1982) except that: (1) the base medium was RPMI 1640 (Grand Island Biological Co., Grand Island, NY, U.S.A., and (2) in some cultures, the foetal calf serum was replaced with serum autologous to the lymphocyte donor. The ability of subjects' sera to inhibit antibody-dependent cellular cytotoxicity (ADCC) of chicken red blood cells and natural killer (NK) of K562 cells by normal donor lymphocytes was assessed by a ^{51}Cr (New England Nuclear Corp., Boston, MA, U.S.A.) release assay. Assays were modified from standard methods (Garovoy & Carpenter, 1980). Heat-inactivated test serum was added to a final concentration of 11%, and supernatants collected with a Skatron filter collection system (Flow Laboratories, Hamden, CT, U.S.A.). Inhibition was defined as test CPM release less than the control release minus 2 s.d. PHA-induced blast cells from inhibited effectors were used as potential ADCC targets to detect antibodies against histocompatibility antigens. Serum inhibition of E rosettes was assessed by preincubating normal donor lymphocytes for 1 h at 4°C, washing, and testing the cells for rosette formation with washed sheep red blood cells. Typing for HLA antigens was kindly provided by Dr Paul Terasaki. Skin tests for delayed hypersensitivity were also performed with PPD, SK-SD, and mumps antigens. Statistical analysis was performed as previously reported (Schuelke *et al.*, 1982).

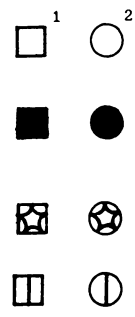
Nineteen individuals represented in Table I and the Figure had intrinsically low blastogenic responses to one or more mitogen when cultured in media containing FCS. Three (III-9, III-29, IV-14) of the 4 living cancer-patients (III-9, III-29, III-38, IV-14) had lowered lymphocyte activity several years after cancer surgery. Benign breast disease in Individual III-39 and advanced age (74 years) in Individual

TABLE I.—*Subjects whose lymphocytes gave responses below the normal range with one or more mitogen in FCS-containing culture medium*

Subject	Medium alone	Mitogen: Con-A[2]	Mitogen: PHA[3]	Mitogen: PWM[4]
II-15	72 ± 45[1]	4439 ± 267	14366 ± 601	3103 ± 376
III-6	54 ± 38	17336 ± 1143	ND[5]	4543 ± 670
III-7	39 ± 19	12608 ± 231	17509 ± 1154	2930 ± 126
III-8	78 ± 22	15508 ± 3712	14926 ± 1578	2417 ± 272
III-9	68 ± 11	14344 ± 1167	16627 ± 817	2785 ± 220
III-10	69 ± 61	23548 ± 1760	25225 ± 6021	3402 ± 114
III-12	93 ± 60	10466 ± 857	5321 ± 366	11293 ± 1178
III-16	99 ± 73	23059 ± 1713	22757 ± 815	3411 ± 71
III-17	10 ± 8	25964 ± 2619	30917 ± 4115	3149 ± 218
III-22	145 ± 13	4524 ± 1365	16509 ± 6477	3482 ± 221
III-29	235 ± 168	21436 ± 1872	14588 ± 2041	14383 ± 254
III-31	101 ± 13	40325 ± 242	13110 ± 2410	14951 ± 1584
III-33	82 ± 63	24959 ± 1024	45895 ± 2645	3605 ± 287
III-34	43 ± 1079	16143 ± 1079	8603 ± 762	13213 ± 2308
III-39	53 ± 32	20560 ± 876	16045 ± 1034	11666 ± 1105
IV-3	117 ± 100	36932 ± 5498	42160 ± 1474	2671 ± 142
IV-9	41 ± 12	38396 ± 2995	32648 ± 3811	3168 ± 106
IV-14	141 ± 114	35936 ± 2697	23262 ± 1087	3184 ± 228

1—Numbers represent mean CPM ± 1 s.d.
2—Normal 95% range = $2{\cdot}675 \times 10^4$–$5{\cdot}166 \times 10^4$ CPM.
3—Normal 95% range = $1{\cdot}866 \times 10^4$–$4{\cdot}929 \times 10^4$ CPM.
4—Normal 95% range = $4{\cdot}07 \times 10^3$–$3{\cdot}868 \times 10^4$.
5—Culture lost due to microbial contamination.

FIGURE



1 2 Code number
Male or female unaffected
Cancer verified by pathology
Multiple primary cancers by family history
Cancer by family history
Proband
Individuals who were Immunologically tested
Individuals with serum IgM > 420 mg/dl
Ov 50 Cancer site and age at diagnosis
d 49 Age at death
63 Age at time of study
(50) mg/dl of serum IgA in those individuals found to be below the 95% range

Sk	Skin
Ov	Ovary
CSU	Cancer site unknown
Ut	Uterus
Le	Leukemia
K	Kidney
GB	Gallbladder
Pr	Prostate
Bl	Urinary bladder
Lu	Lung
Co	Colon
B	Breast
Lym	Lymphoma
OS	Osteogenic Sarcoma
C	Low intrinsic Con-A response
P	Low intrinsic PHA response
W	Low intrinsic PWM response
SC	Autologous serum inhibited Con-A response
SP	Autologous serum inhibited PHA response
SW	Autologous serum inhibited PWM response
NK	Serum inhibited NK killing
AD	Serum inhibited ADCC killing
E	Serum inhibited E rosette formation

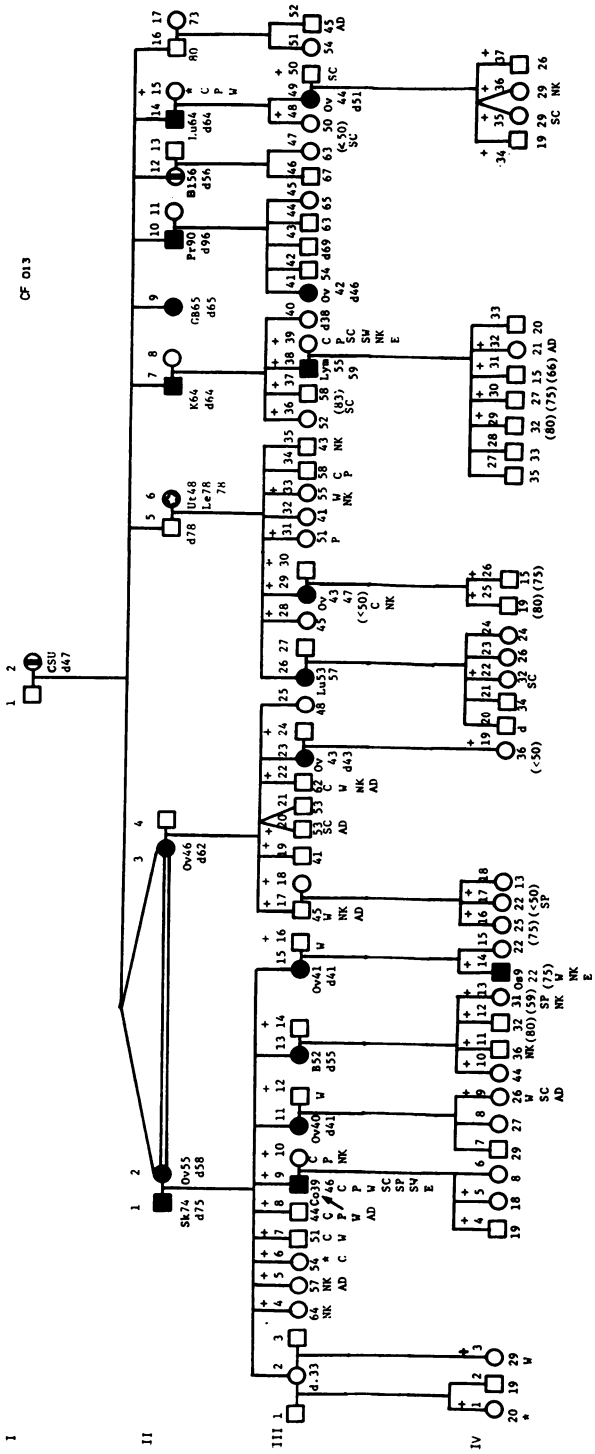

CF 013
I
II
III
IV

TABLE II.—*Subjects whose sera decreased the blastogenic response of autologous lymphocytes to one or more mitogens*

	Serum							
	FCS[1]				Autologous[1]			
Subject	Medium alone	Con-A[2]	PWM	PHA	Medium alone	Con-A	PWM	PHA
III-9	68 ± 11	14344 ± 1167	2785 ± 220	16627 ± 817	126 ± 46	60 ± 18	1432 ± 115	1840 ± 66
III-20	26 ± 25	25982 ± 1772	3776 ± 38	20885 ± 1633	71 ± 61	9702 ± 884	4276 ± 117	29771 ± 897
III-28	68 ± 30	54838 ± 1553	18998 ± 1187	47116 ± 2111	274 ± 198	56813 ± 3698	66866 ± 646	27548 ± 2873
III-36	36 ± 22	50266 ± 7092	6681 ± 373	66527 ± 2235	74 ± 56	26359 ± 3269	5999 ± 287	64301 ± 2489
III-39	53 ± 32	20560 ± 976	11666 ± 1105	16045 ± 1034	51 ± 44	71 ± 23	2303 ± 175	1410 ± 302
III-48	54 ± 32	83003 ± 3811	24478 ± 2917	34658 ± 6977	95 ± 43	62617 ± 1305	52021 ± 2996	36349 ± 3652
III-50	93 ± 25	35346 ± 688	7772 ± 718	46428 ± 2145	276 ± 94	19181 ± 3297	10779 ± 1108	53285 ± 4726
IV-9	41 ± 12	38396 ± 2995	3168 ± 106	32648 ± 3811	134 ± 103	17165 ± 1889	4020 ± 270	49743 ± 2687
IV-13	105 ± 35	52107 ± 4988	14315 ± 1087	56488 ± 5504	68 ± 44	49605 ± 721	26813 ± 1533	38309 ± 3136
IV-17	65 ± 15	37787 ± 4658	12595 ± 1400	30891 ± 2205	346 ± 312	29029 ± 2121	30425 ± 1153	19744 ± 3800
IV-22	82 ± 17	63502 ± 1019	27715 ± 2120	ND	346 ± 213	52418 ± 2815	72206 ± 1835	ND
IV-35	30 ± 13	70988 ± 4232	18875 ± 4232	58595 ± 1865	13 ± 2	30223 ± 1125	22910 ± 3750	53267 ± 1683

1–Lymphocytes stimulated in media containing either FCS or autologous serum.
2–Mean CPM ± 1 s.d. of cultures stimulated with the mitogen indicated. Normal ranges as for Table I.
ND–Not determined.

II-15 were the only other factors identified which might explain the other low responses.

The probabilities of randomly selecting a population with the observed number or a more extreme number of individuals below the normal ranges were calculated to be $P \simeq 0{\cdot}001$, $P \simeq 0{\cdot}014$ and $P \simeq 2 \times 10^{-8}$ for Con-A, PHA, and PWM respectively.

Table II and the Figure show that sera from 12 subjects caused a lower blastogenic response to one or more mitogen, even though the most common observation was unchanged or increased responsiveness. This suppressive activity was evident in one cancer patient (III-9), whose serum suppressed the responses of all 3 mitogens. Inhibitory activity(ies) were also present in 2 spouses (III-39, III-50). The most common selective suppression was of Con-A responses (III-36, III-48, III-50, IV-9, IV-22, IV-35), followed by PHA (III-20, III-28, IV-17), while selective PWM suppression was not observed. A sex-influenced trend for females preferentially to express serum-mediated mitogenic suppression is suggested by the fact that only 3 men (III-9, III-20, III-50) showed such activity, although the sex ratio of the family members sampled was approximately equal.

TABLE III.—*Inhibition of NK effector by family sera**

Serum	%Maximal Cr^{51} release†
Control	$56{\cdot}9 \pm 2{\cdot}6$
III-4	$46{\cdot}5 \pm 0{\cdot}7$
III-5	$42{\cdot}9 \pm 2{\cdot}0$
III-10	$43{\cdot}5 \pm 2{\cdot}7$
III-17	$40{\cdot}2 \pm 0{\cdot}1$
III-22	$40{\cdot}8 \pm 1{\cdot}8$
III-29	$44{\cdot}7 \pm 0{\cdot}5$
III-33	$43{\cdot}5 \pm 3{\cdot}0$
III-35	$43{\cdot}7 \pm 1{\cdot}0$
III-39	$49{\cdot}4 \pm 0{\cdot}1$
IV-11	$47{\cdot}6 \pm 1{\cdot}3$
IV-13	$39{\cdot}8 \pm 2{\cdot}0$
IV-14	$44{\cdot}3 \pm 1{\cdot}7$
IV-36	$42{\cdot}4 \pm 1{\cdot}7$

* Only the data on inhibitory sera are presented. The other sera did not exceed control values by 2 s.d.

† Data presented as average % release ± s.d. Percentage release was calculated by the formula:

$$\% \text{ release} = \frac{ER - SR}{MR - SR} \times 100$$

Where ER = experimental release, MR = maximal release, and SR = spontaneous release.

Serum-mediated inhibition of NK activity (Table III and the Figure) was presented in 11 bloodline individuals and 2 spouses (III-10, III-39). NK inhibitory activity occurred both concomitantly with and independently of ADCC suppressor activity, and was present in the 2 cancer patients with lower serum IgA (III-29, IV-14). Sera from 2 apparently well generation IV females (IV-13, IV-36) also inhibited NK activity.

The Figure shows the 8 bloodline individuals (III-5, III-8, III-17, III-20, III-22, III-52, IV-9, IV-32) whose sera inhibited ADCC effector activity. None of these sera had demonstrable antibodies against the effector cells. Inhibitory activity was present in 2 younger generation IV women (IV-9, IV-32). Based on the results of sera samples from 300 midwestern residents without a known ovarian cancer propensity in their families, it was predicted that 1 in 50 sera would be inhibitory. Thus the probability of randomly selecting a population with 8 or more out of 50 individuals having the activity is calculated to be $P \simeq 3{\cdot}2 \times 10^{-4}$ (Fisher's exact test).

Three sera inhibited E-rosette formation by normal donor T lymphocytes (III-9, III-39, IV-14). Two sera were from cancer-affected individuals (III-9, IV-14) and one was from a spouse (III-39) subsequently proven to have benign breast disease yet who underwent mastectomy. Direct assessment of E-rosette formation by subjects' lymphocytes in the absence of serum failed to reveal any abnormalities not attributable to noncancerous conditions.

No simple association was evident between any single significant abnormal immune finding and blood markers (ABO, Duffy, HLA, or Rhesus antigens) present in the kindred. Skin test results were unremarkable.

Any close association between an im-

munological parameter (or parameters) and a cancer-prone genotype would aid immeasurably in genetic counselling and decision logic for surveillance and management. Long-term follow-up will obviously be needed to determine if any immune parameter(s) identified in cancer families are a constitutionally integral trait correlating with cancer-predisposing genotype and eventual phenotypic expression (clinical cancer).

The immune findings in spouses (III-10, III-12, III-39 and III-50) could represent either random findings unrelated to cancer or a communicable environmental effect (Byers *et al.*, 1975; Dean *et al.*, 1979; Dworsky *et al.*, 1978; Graham-Pole *et al.*, 1976; Guirgis *et al.*, 1978; Hersey *et al.*, 1979). The following observations are consistent with an association of immune findings with cancer-prone genotype: (1) predominance of mitogen suppressive activity in sera from high-risk females; (2) instances of selective mitogen inhibition by family sera similar to hepatocellular carcinoma sera effects on mitogen responses (Ren & Chan, 1981) and (3) increased frequency of ADCC and/or NK serum inhibitory activity in older generation III individuals and selective inhibition of one killing activity by some sera is strikingly similar to the results of a mouse tumour study (Nair *et al.*, 1980). Limitations in sample size and the findings in a single family restrict a more complete elucidation of immunological mechanisms. Logistic and other considerations have to date prevented more extensive immunological analysis than the assays performed as reported above. However, we feel that these data are sufficiently suggestive to warrant further investigations of immune function in other ovarian cancer-prone kindreds in an attempt to identify both a precancerous marker (or markers) and to define further possible aetiological relationships between immune functions and hereditary cancer.

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