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., 1980a, b; Mikulski et al., 1977; Pattillo et al., 1979a, 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 | Con-A² | PHA² | PWM¹ |
|---------|-------------|--------|------|------|
| II-15   | 72 ± 45¹    | 4439 ± 267 | 14366 ± 601 | 3103 ± 376 |
| III-6   | 54 ± 38     | 17336 ± 1143 | ND⁵ | 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 | 16227 ± 817  | 2785 ± 220 |
| III-10  | 60 ± 61     | 23548 ± 1780 | 25225 ± 6021 | 3402 ± 114 |
| III-12  | 93 ± 60     | 10466 ± 857  | 5321 ± 386   | 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     | 24850 ± 1024 | 45805 ± 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 = 2-675 x 10⁴-5-166 x 10⁴ CPM.
3—Normal 95% range = 1-866 x 10⁴-4-929 x 10⁴ CPM.
4—Normal 95% range = 4-07 x 10⁴-3-868 x 10⁴.
5—Culture lost due to microbial contamination.
IMMUNITY IN A CANCER PRONE KINDRED


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

| Subject | Medium alone | Con-A² | PWM | PHA | Medium alone | Con-A | PWM | PHA |
|---------|--------------|--------|-----|-----|--------------|-------|-----|-----|
| III-9   | 68 ± 11      | 14344 ± 1167 | 2785 ± 320 | 16627 ± 817 | 126 ± 46 | 60 ± 18 | 1432 ± 115 | 1840 ± 66 |
| III-20  | 26 ± 25      | 25982 ± 1772 | 3776 ± 38 | 20885 ± 1033 | 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  | 30 ± 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 | 28813 ± 1533 | 38309 ± 3168 |
| IV-17   | 65 ± 15      | 37787 ± 4688 | 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 1.
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 $P \approx 0.001$, $P \approx 0.014$ and $P \approx 2 \times 10^{-8}$ 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.

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 $P \approx 3.2 \times 10^{-4}$ (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-

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**Table III.** Inhibition of NK effector by family sera

| Serum | %Maximal Cr$^{51}$ release$^\dagger$ |
|-------|-------------------------------------|
| Control | 56.9 ± 2.6 |
| III-4 | 46.5 ± 0.7 |
| III-5 | 42.9 ± 2.0 |
| III-10 | 43.5 ± 2.7 |
| III-17 | 40.2 ± 0.1 |
| III-22 | 40.8 ± 1.8 |
| III-29 | 44.7 ± 0.5 |
| III-33 | 43.5 ± 3.0 |
| III-35 | 43.7 ± 1.0 |
| III-39 | 49.4 ± 0.1 |
| IV-11 | 47.6 ± 1.3 |
| IV-13 | 39.8 ± 2.0 |
| IV-14 | 44.3 ± 1.7 |
| IV-36 | 42.4 ± 1.7 |

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

$\dagger$ 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.
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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