CHAPTER 19

IMMUNOLOGICAL EVALUATION

INTRODUCTION

Overt damage to organs of the immune system and depressed immunologic function have been noted in a variety of animals exposed to TCDD. As the fields of immunology and immunotoxicology have grown within the past 10 years, a significant spectrum of subtle immunotoxic effects has also been described in animals, but for many possible reasons, comparable adverse effects have not been consistently recorded in exposed human individuals or cohorts.

Thus, an intensive search is underway to ascertain the effects of TCDD on the human immune system, particularly with respect to the development of cancer. Every major ongoing dioxin morbidity study in the United States has now incorporated comprehensive laboratory assessments of the immune system.

Numerous animal studies have demonstrated significant immunotoxicity following the administration of TCDD. The relatively consistent observations of decreased thymus weight (with cortical atrophy and a depletion of lymphocytes), atrophy of other lymphoid tissue, depressed cellular bone marrow, and decreased humoral and cell-mediated immunity and increased susceptibility to infection have been noted in a variety of animals, including monkeys, rabbits, guinea pigs, rats, and mice.[1-12] The immune-response effects varied by species, species strain, age, integrity of the endocrine system, dose, and route of administration. Generally, the immunologic parameters returned to normal or approximate normal values over time, even following moderate to high doses of TCDD. While experiments have been conducted to assess the immunotoxicity of TCDD, little has been published on the immunotoxicity of 2,4-D or 2,4,5-T.

The immune system is so sensitive to TCDD that immune function has frequently been used as a marker of toxicity in the absence of other biologic effects.[13] The mechanism of TCDD immunotoxicity is under intensive investigation by molecular biologists, pathologists, and geneticists. In general, TCDD toxicity is probably linked to the Ah receptor, and specifically to the Ah^b allele which governs microsomal enzyme induction as reflected by aryl hydrocarbon hydroxylase and cytochrome P-448/450 levels.[14] This premise underscores the questions of the degree to which the human response to TCDD is dependent upon the Ah locus or other genetic receptors, and how this response is manifested in the immune system.

Animal studies and several observational studies in humans have shown variable results. Data from the Times Beach, Missouri, episode disclosed no group differences for various T lymphocyte populations, proliferative responses to PHA, concanavalin A, pokeweed or tetanus toxoid stimulation, and in skin testing with seven antigens.[15] A report of the assessment of the immune system of men exposed to TCDD in an industrial accident in Britain

did not discuss the results of the measurement of the immunoglobulin profile, lymphocytes, T and B cells, response to PHA, and three hematologic variables.[16] A prior publication on the same cohort cited unpublished findings of Ward, suggesting a reduction in the capacity of the "primary" immune system.[17]

A longitudinal evaluation of 48 highly exposed children (one-half with chloracne) from the Seveso incident showed significantly elevated complement hemolytic activity over six measurements during the period 1976 to 1979 (although the biologic significance of this is unknown) and an increased proliferative response to PHA and pokeweed during the first three screenings.[18] This study (as others) was characterized by shifting a study population over the observation period and by excessive laboratory variation that may have masked other true group differences. Nonetheless, the Seveso data may be interpreted as indicative of a stimulated immune system, particularly cell-mediated immunity, differing substantially from the bulk of animal studies, which showed decreased activity.

A recent study of residents of the TCDD-contaminated Quail Run Mobile Home Park in Missouri also revealed data that conflicted with the Seveso experience.[19] A statistically significant amount of anergy and relative anergy was detected in the TCDD-exposed group, as determined by the multitest applicator (seven-antigen test system). Inter-reader variation presented major interpretive difficulties. Nevertheless, findings suggestive of decreased cell-mediated immunity were provided by decreased T_3, T_4, and T_{11} cell percentages. Also noted was an increased lymphoproliferative response to pokeweed mitogen (PWM). The overall depression of immunologic response was not correlated with an increase in clinical disease.

Baseline Summary Results

Immunologic function and phenotypic marker studies were performed on 592 participants (297 Ranch Hands, 295 Comparisons) randomly selected by the terminal digit of their case number. Because of laboratory problems, e.g., fluctuating quality control and lack of simultaneous differential counts on the peripheral mononuclear cells, a special Immunology Review Committee was convened to determine which data were relevant for analysis. Such decisions were made on a case-by-case basis without knowledge of Ranch Hand or Comparison group membership. The Committee concluded that the data could be analyzed on a group basis, but interpretation of data on an individual basis was inappropriate.

Analyses of the cell surface markers (T_{11}, T_3, T_4, T_8, B, the T_4/T_8 ratio, and the total lymphocyte count [TLC]) showed no significant group differences. However, the increased smoking was significantly associated with increases in cell counts but not with the T_4/T_8 ratio and B_1 cells, whereas increasing age was significantly associated with decreasing TLC and T_8 cells.

Functional studies of T and B cells via reaction to antigenic (tetanus toxoid) or mitogen (phytohemagglutinin, concanavalin A, and pokeweed) stimulation showed no group differences. Similarly, unadjusted and adjusted mean values of the four assays were not significantly different between groups, but one unstimulated control value (reflecting Baseline thymidine uptake by T cells) was significantly decreased in the Ranch Hands. The biologic relevance of this finding was unclear.

Further, in the covariate analysis of the functional studies, group-by-smoking and group-by-alcohol interactions were noted. Of greater importance, however, was the finding that lymphocytic response increased with increased smoking, but was depressed in association with increasing age.

In summary, both immunologic function and cell marker studies did not show significant impairment in the Ranch Hand group, or patterns supportive of an herbicide effect. Smoking, for the first time to the knowledge of the authors, was associated with a significant increase in the marker cells T_{11}, T_3, T_4, and T_8, and in the total lymphocyte count, with a concomitant increase in lymphocytic response to PWM.

Parameters of the 1985 Immunologic Profile

The format for the 1985 AFHS physical examination placed more emphasis on the immunologic assessment than did the 1982 Baseline profile. The random sampling scheme was expanded to produce an approximate 50-percent sample of the cohorts, and included the same terminal digits of the case number used at Baseline in order to include all individuals evaluated in 1982 and thus establish a longitudinal data base.

All immunologic tests were performed at the Scripps Immunology Reference Laboratory (SIRL). The battery of phenotypic marker assays and functional tests was slightly modified from the Baseline profile. The assay for HLA-DR cells was added to the battery of marker studies, and a functional test for natural killer cells (with and without interferon) was substituted for the concanavalin A stimulation assay. A comprehensive set of skin tests for the antigens Candida, mumps, Trichophyton, and staph-phage-lysate was added to evaluate the integrity of the delayed hypersensitivity response.

The dependent variables of the analyses in this chapter arise from three distinct measurement systems: phenotypic marker studies, functional studies, and skin testing. There were more covariates than in the Baseline study, namely age, race, occupational category, exposure index, and new smoking and alcohol data from the 1985 questionnaire.

Participants deleted from the immunological analyses included those with recent radiation or chemotherapy, and those individuals on immunosuppressive or systemic steroid medication. Marginal totals in the tables below vary somewhat due to missing covariate data. Thus, numbers in the table also vary according to which immunologic data sources were used in the analysis. In general, most analyses are based upon data from 465 Ranch Hands and 585 Comparisons. Analytic tests included t-tests, general linear models (SAS®-GLM), logistic regression (BMDP®-LR), and Fisher's exact test. Parallel analyses using Original Comparisons are in Tables Q-5 through Q-10 of Appendix Q.

Rationale of the Immunologic Measurements

Because of rapid changes in our knowledge of the immune system, Table 19-1 is provided as an aid in interpreting the medical significance of the immunological data.

TABLE 19-1.

Medical Significance of the Immunological Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
MARKER STUDIES		
OKT_{11}	Measures total T cells coincident with sheep rosette receptor on cell surface (most are T_4 and T_8 cells).	Decreased in immune deficiency/ increased with lymphoproliferative disorders.
Leu 12	Measures peripheral blood B cells, no reaction with T cells, granulocytes, or monocytes.	Decreased in immunodeficiency/ increased in lymphoproliferative disorders.
OKT_4	Measures T cells which exhibit helper/ inducer phenotype.	Decreased in AIDS/increased in autoimmune diseases.
OKT_8	Measures T cells which exhibit suppressor/ cytotoxic functions.	Variable in autoimmune diseases. Increased in some viral illnesses and immunodeficiencies.
Leu M3	Measures mature monocytes in peripheral blood.	Increases with inflammation.
HLA-DR	Measures cells expressing HLA-DR antigen; includes B cells and monocytes.	B cell deficiency/ Agammaglobulinemia.
FUNCTIONAL STUDIES		
Mixed Leukocyte Culture (MLC)	Measures reactivity of T cells to foreign histocompatiblity antigens on unrelated lymphocytes.	HLA sensitization/transplantation.
PHA	Measures functional capability of T cells to become activated by mitogen and undergo proliferation.	T cell deficiency.
NKC (with interferon) NKC (without interferon)	Measures natural killer cell lytic activity with and without interferon treatment of the natural killer cells.	Decreased natural defenses.

TABLE 19-1. (continued)

Medical Significance of the Immunological Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
FUNCTIONAL STUDIES (continued)		
PWM	Measures functional capability of T cells to become activated by mitogen and undergo proliferation.	T cell deficiencies.
SKIN TESTS		
Candida Mumps Tricophyton Staph-phage-lysate	Each measures skin reactivity induced by specific antigen injected intradermally and correlates with recall T cell sensitivity to the antigen.	Antigen reactivity or sensitivity/ Anergy.

Immunology Methodologies

The isolation of peripheral blood mononuclear (PBM) cells was the first step to prepare for testing immune competence and enumeration of phenotypic markers. Heparinized whole blood was obtained from each patient. PBM's were isolated by Ficoll-Hypaque density gradient centrifugation. The PBM's were then washed and resuspended in HB101 media (HANA Biologics, Inc.) supplemented with 10M units/ml penicillin, 10,000 mcg/ml streptomycin, 1 percent sodium pyruvate (100 mM), and 1 percent L-glutamine (200 mM). To determine percent monocyte and granulocyte contamination of the PBM cell preparations, an aliquot of the cells was stained with a nonspecific esterase stain. PBM concentration was adjusted for each individual assay.

Cell Surface Marker Analysis

Mouse monoclonal antibodies directed against specific surface markers were used to identify and quantitate different cell populations in the peripheral blood of the participants. Mononuclear cell concentrations adjusted to 1.0×10^6 cells/ml were incubated with the following fluorescein isothiocyanate conjugated monoclonal antibodies: CD2(OKT11*), CD4(OKT4*), CD8(OKT8*), CD19(Leu12**), CD14(LeuM3**), and HLA-DR(OKDR*). These cell surface antibodies measure total numbers of T and B lymphocytes, monocytes, helper T lymphocytes, suppressor T lymphocytes, and those cells carrying the HLA-DR antigen. A flow cytometer (Spectrum III, Ortho Diagnostic Systems, Raritan, New Jersey) was used to measure percent positive for each specific surface marker and absolute numbers were calculated.

Phytohemagglutinin (PHA) and Pokeweed Mitogen Stimulation Assays

Mitogens were used to stimulate the proliferation of lymphocytes in vitro. During the proliferative response, the lymphocytes undergo blast transformation and incorporate radioactive thymidine into their DNA. Participant lymphocyte concentrations were adjusted to 2.0×10^6 cells/ml in supplemented HB101 media. Samples were cultured in quadruplicate. Individual cultures consisted of 0.1 ml of cell suspension and 0.1 ml of mitogen solution in microtiter plates. The cultures were incubated in an atmosphere of 5 percent CO_2 at 37 degrees Centigrade. Participant cells were cultured with PHA (12 µg/ml, Sigma Chemical Co., St. Louis, Missouri) for a total of 4 days and pokeweed mitogen (0.05 µg/ml, Sigma Chemical Co., St. Louis, Missouri) for a total of 5 days. The cultures were pulsed with tritiated thymidine (1.0 µCi/ microtiter well) for 4 hours and then harvested on a multiple automated harvester. Cellular proliferation was assessed by tritiated thymidine uptake measured by liquid scintillation counting.

Mixed Lymphocyte Reaction

Histocompatibility antigens (HLA) can also stimulate lymphocytes causing blast transformation. Donor lymphocytes were used to stimulate the proliferation of participants' lymphocytes in vitro. A pool of donor lymphocytes

*Ortho Diagnostic Systems, Raritan, New Jersey
**Becton Dickinson Monoclonal Center, Inc., Mountain View, California

was frozen and used as a stimulator pool throughout the course of the study. An aliquot of this pool was thawed daily. Viability of this pool was assessed by trypan blue exclusion. A pool of freshly isolated lymphocytes was prepared daily and also used as stimulator cells. Both pools of stimulator cells were inactivated by irradiation (3,000 rad). Stimulator pools and participant lymphocyte concentrations were adjusted to 1.0×10^6 cells/ml in supplemented HB101 media. Samples were cultured in quadruplicate. Individual cultures consisted of 0.1 ml of participant cell suspension and 0.1 ml of stimulator cell suspension, in microtiter plates. The cultures were incubated in an atmosphere of 5 percent CO_2 at 37 degrees Centigrade for 6 days. The cultures were pulsed with tritiated thymidine (1.0 µCi/ microtiter well) for 16 hours and then harvested on a multiple automated cell harvester. Cellular proliferation was assessed by tritiated thymidine uptake measured by liquid scintillation counting.

Natural Killer Cell Assays

Mononuclear cells from the participant were evaluated to assess the ability of certain peripheral blood cells to kill target cells from a K-562 leukemia cell line. The K-562 target cells were preincubated with radioactive chromium (^{51}Cr) at 37 degrees Centigrade in 5 percent CO_2 for 1 hour, washed, and the cell concentration adjusted to 1.6×10^5 cells/ml. A 50 µl aliquot of radioactive K-562 cells was added to each microtiter well. Participant lymphocytes were adjusted to three different concentrations: 0.53, 1.6, and 2.7×10^6 cells/ml. One ml of each of these concentrations was incubated with 20 units of recombinant γ-interferon (Genentech, Inc., San Francisco, California) for 1 hour at about 37 degrees Centigrade. Quadruplicate 150 µl aliquots of each concentration, with and without interferon preincubation, were dispensed in a microtiter plate. Four wells contained media alone to determine the spontaneous release of radioactivity from the K-562 cells. Four wells contained 1 percent Triton X-100 to determine the maximal release of radioactivity. The final effector to target ratios were 50:1, 30:1, and 10:1. The microtiter plates were centrifuged briefly at low speed and incubated at 37 degrees Centigrade in 5 percent CO_2 for 3 hours. A 100 µl aliquot of the supernatant was removed from each well and counted on a gamma counter. Percent chromium release from the K-562 target cells was determined for each effector:target cell ratio.

Interpretive Considerations

The values of the results of assays of immunologic status are more variable than those found in routine single reactant clinical chemistry assays. Often there are numerous biochemical factors/metabolites that affect the immunologic assay results so that interpretations of normalcy must be in the context of those obtained concurrently in a normal control cohort group. Such controls allow for proper adjustments of the raw assay data in order to minimize the broad range of technical and reagent effects in the various immunologic assays. These adjustments in the raw assay data results will correct for such variability and allow for the detection of any significant biologic abnormalities. Because of the need for these control adjustments, the immunologic assay results cannot be meaningfully compared to existing normal ranges determined on different groups of individuals at other times.

RESULTS AND DISCUSSION

Cell Surface Marker (Phenotypic) Studies

Immunological tests were carried out on 47 percent (1,085) of the participants because of the complexity of the assay and the expense of these tests. The participants were randomly selected so that approximately 50 percent of each group of participants arriving for the physical examination had blood drawn for the immunological tests. Logistical delay during the initial weeks of the examination reduced the number to less than 50 percent. Within each group, blood was drawn for the immunological tests from about one-half of the selected participants on the first day of the physical examination, and from the remainder on the following day. Skin tests, which were scheduled for the first day, were therefore carried out after the blood draw on the first day for the first half of the immuno-tested participants. Skin tests were not done for those participants selected for immunological testing on day two in order to avoid any effect the skin test antigens might have on the cell counts and functions. Thus, 553 participants received both the immunological tests and the skin tests, 532 received the immunological tests but not the skin tests, 1,206 received the skin tests but not the immunological tests, and 18 received neither. Table 19-2 gives the frequencies of the participants in each exposure group who had the tests.

Participants who were taking anti-inflammatory or immunosuppression medication or who had recently received x-ray treatment or chemotherapy for cancer were excluded from all the analyses. Participants taking aspirin, however, were not excluded.

TABLE 19-2.

Frequencies of Participants Who Took the Immunological Tests and the Skin Tests, by Group

Group	Immunology Tests	Skin Tests: No	Skin Tests: Yes	Skin Tests: Total
Ranch Hand	No	9	524	533
	Yes	218	265	483
	Total	227	789	1,016
Comparison	No	9	682	691
	Yes	314	288	602
	Total	323	970	1,293

For those participants who were given the immunological tests, the following dependent variables were examined: total T cells, helper T cells, suppressor T cells, B cells, monocytes, HLA-DR cells, and the T_4/T_8 (helper/suppressor cell) ratio. These variables were treated as continuous in the analysis.

The covariates considered in the analysis were the matching variables (age, race, occupation), smoking history (current cigarettes/day and total pack-years of smoking), and alcohol consumption (average number of drinks per day during the 2 weeks prior to the physical examination and total drink-years). The covariates age and the smoking history and alcohol consumption variables were used as continuous variables in the analyses since the relationships between the dependent variables and the covariates were generally monotonic.

Considerable day-to-day variation exists in the results of immunological tests due to a number of extraneous factors, including temperature, humidity, and sensitivity of the instrumentation. Significant batch-to-batch variation (among examination groups) was apparent for total T cells, suppressor T cells, B cells, and the T_4/T_8 ratio, and significant blood-draw day variation was apparent for helper T cells, monocytes, and HLA-DR cells. Adjustments in the analyses were made for these sources of variation by using batch or blooddraw day indicators. Throughout this section, appropriate adjustment was carried out in the assessment of group differences of the dependent variables; this analysis was unadjusted for the covariates listed above and is referred to as the "unadjusted" analysis. Adjustment was also made for batch-to-batch or blood-draw day variation in the analyses of the associations of the dependent variables with the covariates. Further, this adjustment was also used in the fitting of general linear models to assess the group differences, adjusted for the covariates.

Prior to analysis, group data were pooled for each continuous variable and were examined to determine whether transformation would enhance normality or distributional symmetry. The following transformations were used in the analyses:

Variable	Transformation
Total T cells	Square root
Helper T cells	Square root
Suppressor T cells	Logarithm
B cells	Square root
Monocytes	Logarithm
HLA-DR cells	Square root
T_4/T_8 ratio	Logarithm

The results of the analyses in this section are summarized in Tables 19-3 through 19-5. Table 19-3 presents the unadjusted analyses for the cell surface markers, Table 19-4 displays the covariate associations, and Table 19-5 gives the adjusted results. These tables are accompanied by

TABLE 19-3.

Unadjusted Analyses for Cell Surface Markers by Group

Variable	Statistic	Group		p-Value
		Ranch Hand	Comparison	
Total T Cells (T_{11})	n	464	581	
	Mean	1,616	1,604	0.736
	95% C.I.	(1,561, 1,671)	(1,556, 1,653)	
Helper T Cells (T_4)	n	461	580	
	Mean	874.6	863.3	0.610
	95% C.I.	(839.9, 909.9)	(833.3, 893.9)	
Suppressor T Cells (T_8)	n	465	582	
	Mean	523.6	530.0	0.671
	95% C.I.	(500.1, 548.1)	(508.9, 552.0)	
B Cells	n	457	575	
	Mean	185.6	189.5	0.594
	95% C.I.	(174.1, 197.4)	(179.2, 200.1)	
Monocytes	n	462	582	
	Mean	46.08	44.49	0.427
	95% C.I.	(42.99, 49.39)	(41.88, 47.27)	
HLA-DR Cells	n	462	582	
	Mean	571.4	568.4	0.842
	95% C.I.	(547.9, 595.3)	(548.1, 589.1)	
T_4/T_8 Ratio	n	461	577	
	Mean	1.600	1.570	0.499
	95% C.I.	(1.531, 1.672)	(1.510, 1.633)	

TABLE 19-4.

Association Between Cell Surface Marker Variables and the Covariates in the Combined Ranch Hand and Comparison Groups (Directionality Shown)

Variable	Race	Occupation	Age	Current Alcohol (Drinks/Day)	Drink-Years	Current Smoking (Cigarettes/Day)	Lifetime Smoking (Pack-years)
Total T Cells	NS	0.005 O<E	0.002[a]	0.069[a]	NS	<0.001[b]	<0.001[c]
Helper T Cells	NS	0.024 O<E	<0.001[a]	NS	NS	<0.001[b]	<0.001[c]
Suppressor T Cells	NS	<0.001 O<G<F	<0.001[a]	0.058[d]	NS	<0.001[e]	0.076[c]
B Cells	NS	<0.001 O<E	<0.001[a]	0.001[a]	0.047[f]	<0.001[e]	0.005[c]
Monocytes	0.027 N>B	0.019 O<F<G	NS	0.031[g]	<0.001[h]	<0.001[c]	<0.001[c]
HLA-DR Cells	NS	<0.001 O<E	0.010[a]	NS	0.083[c]	<0.001[c]	<0.001[c]
T_4/T_8 Ratio	NS	0.063 F<G<O	NS	NS	0.049[c]	<0.001[c]	0.001[c]

[a]Monotone decreasing.

[h]Increases, drop-off at highest category.

[c]Monotone increasing.

[d]Generally decreasing trend.

[e]Increases from 0 category, then decreases, but not back to same level.

[f]Increases from 0 category, then steady decrease with increasing levels.

[g]Generally increasing trend.

[h]Flat for 0 and first few categories, then increases.

NS: Not significant (p>0.10)
N: Nonblack
B: Black
O: Officer
E: Enlisted personnel (flyer and groundcrew)
F: Enlisted flyer
G: Enlisted groundcrew

TABLE 19-5.

Adjusted Analyses for Cell Surface Markers by Group

Variable	Statistic	Group: Ranch Hand	Group: Comparison	p-Value	Covariate Remarks*
Total T Cells	n	442	567		BATCH (p=0.029)
	Adj. Mean	****	****	****	AGE (p=0.009)
	95% C.I.	****	****		ALC (p=0.001)
					CSMOK (p<0.001)
					GRP*RACE (p=0.033)
					DRKYR*PACKYR (p=0.015)
Helper T Cells	n	439	566		BATCH (p=0.021)
	Adj. Mean	869.4	878.5	0.662	DAY(BATCH) (p=0.014)
	95% C.I.	(836.1, 903.2)	(849.2, 908.4)		AGE (p<0.001)
					ALC*OCC (p=0.008)
					CSMOK*OCC (p=0.023)
					ALC*CSMOK (p=0.006)
					DRKYR*PACKYR (p=0.012)
Suppressor T Cells	n	463	580		BATCH (p<0.001)
	Adj. Mean	530.8	537.9	0.640	OCC (p=0.014)
	95% C.I.	(506.8, 556.0)	(516.1, 560.5)		AGE (p=0.004)
					ALC (p=0.020)
					CSMOK (p<0.001)
B Cells	n	435	561		BATCH (p<0.001)
	Adj. Mean	****	****	****	ALC (p=0.006)
	95% C.I.	****	****		AGE*CSMOK (p=0.025)
					DRKYR*RACE (p=0.026)
					GRP*PACKYR (p=0.018)
					GRP*RACE*OCC (p=0.046)
Monocytes	n	440	568		BATCH (p<0.001)
	Adj. Mean	****	****	****	DAY(BATCH) (p<0.001)
	95% C.I.	****	****		RACE (p=0.032)
					DRKYR (p=0.013)
					CSMOK (p<0.001)
					PACKYR (p=0.006)
					GRP*OCC (p=0.044)
					GRP*ALC (p=0.010)

TABLE 19-5. (continued)

Adjusted Analyses for Cell Surface Markers by Group

Variable	Statistic	Group: Ranch Hand	Group: Comparison	p-Value	Covariate Remarks*
HLA-DR Cells	n	459	580		BATCH (p<0.001)
	Adj. Mean	****	****	****	DAY(BATCH) (p=0.004)
	95% C.I.	****	****		OCC (p=0.035)
					CSMOK (p<0.001)
					GRP*ALC (p=0.045)
					AGE*PACKYR (p=0.005)
T_4/T_8 Ratio	n	461	577		BATCH (p<0.001)
	Adj. Mean	1.570	1.552	0.678	OCC (p=0.020)
	95% C.I.	(1.501, 1.643)	(1.491, 1.616)		CSMOK (p<0.001)

*Abbreviations
BATCH: batch-to-batch variation among examination groups
DAY(BATCH): blood-draw day variation
ALC: current alcohol use
CSMOK: current smoking
OCC: occupation
GRP: group
DRKYR: lifetime alcohol use (drink-years)
PACKYR: lifetime smoking (pack-years)

****Significant group-by-covariate interaction--adjusted mean, confidence interval, and p-value not presented.

discussion of each variable. The results of adjusted analyses with group-by-covariate interactions are found in Table Q-1 of Appendix Q.

Total T Cells (T_{11})

No significant difference was found between groups in the mean values of total T cells (p=0.736). These data were analyzed without adjustment for any covariates except batch-to-batch variation.

The data were pooled for the two groups, and the relationship with the covariates was examined. Significant associations were found with occupation (p=0.005), age (p=0.002), current smoking (p<0.001), and pack-years (p<0.001). A marginal association (p=0.069) was found with current alcohol use due to a steady decrease in mean counts with higher drinking levels. Officers had a lower mean count (1,539 cells/mm^3) than enlisted flyers (1,668 cells/mm^3), or enlisted groundcrew (1,647 cells/mm^3). The mean count decreased with age: 1,663 cells/mm^3, 1,582 cells/mm^3, and 1,404 cells/mm^3 for those born in or after 1942, born between 1923 and 1941, and born in or before 1922, respectively. The mean count increased with increasing current smoking and increasing lifetime smoking history (pack-years).

A general linear model was fitted to assess the group difference in mean count of total T cells with adjustment for each covariate and any interactions that made significant contributions to the model. Batch-to-batch variation was a significant covariate (p=0.029).

A significant group-by-race interaction was found (p=0.033); Black Ranch Hands had a significantly lower adjusted mean count than Black Comparisons (1,566 cells/mm^3 versus 1,888 cells/mm^3; p=0.039), but the group difference for nonblacks was not significant (p=0.619) (see Table Q-1 of Appendix Q). The following covariates were significant: age (p=0.009), current alcohol use (p=0.001), current smoking (p<0.001), and a drink-year-by-pack-year interaction (p=0.015). Analyses using only Original Comparisons showed the same results as when using the total Comparison group (see Tables Q-6 and Q-7 of Appendix Q), with a group-by-race interaction present (p=0.028).

Helper T Cells (T_4)

No significant difference was found between groups in the mean values of helper T cells (p=0.610). This contrast was analyzed without adjustment for any covariates except blood-draw day variation.

The data were pooled for the two groups, and the relationship with the covariates was examined. Significant associations were found with occupation (p=0.024), age (p<0.001), current smoking (p<0.001), and pack-years (p<0.001). Officers had a lower mean count (831 cells/mm^3) than enlisted flyers (885 cells/mm^3) or enlisted groundcrew (894 cells/mm^3). There was a decrease in the mean count with increasing age: 907 cells/mm^3, 850 cells/mm^3, and 713 cells/mm^3 for those born in or after 1942, born between 1923 and 1941, and born in or before 1922, respectively. The mean count increased with increasing levels of current smoking and with increasing pack-years of lifetime smoking.

Adjusted analyses assessed the group difference in mean count of helper T cells with adjustment for each covariate and any significant interactions. Adjustment for the blood-draw day variation was included. Age made a significant contribution to the model ($p<0.001$). The following interactions between covariates were significant: current alcohol use-by-occupation ($p=0.008$), current smoking-by-occupation ($p=0.023$), current alcohol use-by-current smoking ($p=0.006$), and drink-years-by-pack-years ($p=0.012$). The adjusted group difference in mean count was not significant ($p=0.662$): 869 cells/mm^3 for the Ranch Hand group versus 879 cells/mm^3 for the Comparison group. Adjusted analyses using Original Comparisons (Table Q-6 of Appendix Q) also revealed a nonsignificant group difference ($p=0.835$).

Suppressor T Cells (T_8)

No significant difference was found between groups in the mean values of suppressor T cells ($p=0.671$). This contrast was analyzed without adjustment for any covariates except batch-to-batch variation.

The data were pooled for the two groups, and the relationship with the covariates was examined. Significant associations were found with occupation ($p<0.001$), age ($p<0.001$), and current smoking ($p<0.001$). The mean count for officers was less than the mean count for enlisted groundcrew, which was in turn less than the mean count for enlisted flyers; the means were 492 cells/mm^3, 540 cells/mm^3, and 575 cells/mm^3, respectively. The mean counts decreased with increasing age: 557 cells/mm^3, 512 cells/mm^3, and 439 cells/mm^3 for participants born in or after 1942, born between 1923 and 1941, and born in or before 1922, respectively. The mean counts increased with increasing levels of current smoking. Marginally significant associations were found with current alcohol use ($p=0.058$, mean T_8 counts decreased with increasing current levels of drinking) and pack-years ($p=0.076$, mean counts increased with increasing pack-years).

The adjusted analysis of group differences in mean count of suppressor T cells was made with adjustment for each covariate and any interactions that made significant contributions, including significant batch-to-batch variation ($p<0.001$). Significant adjusting covariates were occupation ($p=0.014$), age ($p=0.004$), current alcohol use ($p=0.020$), and current smoking ($p<0.001$). The adjusted group difference was not significant ($p=0.640$).

A marginal ($p=0.063$) group-by-race interaction was not retained in the final model, but was explored. Black Ranch Hands had a lower adjusted mean count than Black Comparisons (512 cells/mm^3 versus 649 cells/mm^3, $p=0.056$), whereas the difference between nonblack groups was negligible (531 cells/mm^3 for Ranch Hands and 532 cells/mm^3 for Comparisons, $p=0.974$). Analyses involving the Original Comparisons showed a significant interaction between group and race ($p=0.010$) (see Tables Q-6 and Q-7 of Appendix Q), with the same pattern seen for the group-by-race interaction for the total Comparison group.

B Cells

No significant difference was found between groups in the mean values of B cells ($p=0.594$). This contrast was analyzed without adjustment for any covariates except batch-to-batch variation.

Significant associations using pooled data were found between B cells and occupation ($p<0.001$), age ($p<0.001$), current alcohol use ($p=0.001$), drink-years ($p=0.047$), current smoking ($p<0.001$), and pack-years ($p=0.005$). Officers had a lower mean count than enlisted flyers and groundcrew (166 cells/mm^3, 205 cells/mm^3, and 201 cells/mm^3, respectively). The mean count decreased with increasing age: 206 cells/mm^3, 177 cells/mm^3, and 146 cells/mm^3 for those born in or after 1942, born between 1923 and 1941, and born in or before 1922, respectively. The mean counts decreased with an increasing number of drinks per day, and also with higher levels of total lifetime drinking, except for the "never-drinkers," whose level was lower than the greater than 30 to 100 drink-year group; the means for the drink-year categories were: 0, 180 cells/mm^3; greater than 0 to 5, 199 cells/mm^3; greater than 5 to 30, 189 cells/mm^3; greater than 30 to 100, 183 cells/mm^3; and greater than 100, 150 cells/mm^3. The nonsmokers had a lower mean count than current smokers, whereas among the smokers the mean counts decreased with higher current smoking levels. The means for the different current-smoking (cigarettes/day) categories were: 0, 166 cells/mm^3; greater than 0 to 20, 237 cells/mm^3; greater than 20 to 40, 222 cells/mm^3; and greater than 40, 202 cells/mm^3. Lifetime smokers had a higher mean count than "never-smokers"; otherwise the pattern was not clear.

Adjusted analyses, including adjustment for the significant ($p<0.001$) batch-to-batch variation, were used to investigate the mean count of B cells. Adjustment was made for each covariate and any interactions that made significant contributions. A significant group-by-race-by-occupation interaction was found ($p=0.046$), along with a group-by-pack-year interaction ($p=0.018$). Significant contributions were made by current alcohol use ($p=0.006$), an age-by-current smoking interaction ($p=0.025$), and a drink-years-by-race interaction ($p=0.026$).

The analysis consequently was performed separately for nonblacks and Blacks. For nonblacks, the group-by-pack-year interaction persisted ($p=0.021$) (see Table Q-1 of Appendix Q). Ranch Hands who had never smoked had a much lower adjusted mean count than the corresponding Comparisons, 154 cells/mm^3 versus 190 cells/mm^3 ($p=0.004$). Among smokers, the adjusted mean count for the greater than 0 to 20 pack-year category was less for Ranch Hands than for Comparisons. For both the greater than 20 to 40 and the greater than 40 pack-year categories, the adjusted mean count was higher for Ranch Hands than for Comparisons. The p-values for these three contrasts were greater than 0.10. For Blacks, the unadjusted group difference was not significant ($p=0.808$; Ranch Hands, 186 cells/mm^3, versus Comparisons, 194 cells/mm^3). Adjusted means were not calculated because no covariates made any significant contribution to an adjusted model, and moreover, adjustment for batch-to-batch variation was not possible because of the small number of Black participants.

Other significant covariates and interactions in the adjustment for nonblacks included occupation ($p=0.047$), drink-years ($p<0.001$), and an age-by-current smoking interaction ($p=0.039$).

Monocytes

No significant difference was found between groups in the mean value of monocytes ($p=0.427$). This contrast was analyzed without adjustment for any covariates except blood-draw day variation.

The data were pooled for the two groups, and the relationship with the covariates was examined. Significant associations were found with race ($p=0.027$), occupation ($p=0.019$), current drinking ($p=0.031$), drink-years ($p<0.001$), current smoking ($p<0.001$), and pack-years ($p<0.001$). Blacks had a lower mean count than nonblacks (37.1 cells/mm^3 versus 45.7 cells/mm^3, respectively). Officers had a lower mean count (42.3 cells/mm^3) than enlisted flyers (44.4 cells/mm^3), who had a lower mean count than enlisted groundcrew (48.2 cells/mm^3). Higher mean counts were associated with higher current drinking levels. There were increases in mean counts with higher drink-years and with increasing amounts of both current and lifetime smoking.

Assessment of the group difference in mean count of monocytes was done with adjustment for each covariate and any interactions that made significant contributions, including blood-draw day variation.

A significant group-by-occupation interaction ($p=0.044$) and a significant group-by-current alcohol use interaction ($p=0.010$) were found. For interpretation, these were explored in a model including the group-by-occupation-by-current alcohol use interaction, with the alcohol variable discretized (see Table Q-1 of Appendix Q). Except for those men consuming more than two to four drinks per day, Ranch Hand officers had a higher adjusted mean count than Comparison officers, the difference being large (44.2 cells/mm^3 versus 32.3 cells/mm^3) for nondrinkers, ($p=0.060$). For enlisted flyers, except those in the greater than four drinks per day category, Ranch Hands had a lower adjusted mean count than corresponding Comparisons. For the greater than two to four drinks per day category, a large difference between adjusted means (32.7 cells/mm^3 for Ranch Hands, 56.2 cells/mm^3 for Comparisons) was observed ($p=0.097$). Further, it was found that for enlisted groundcrew not currently drinking, Ranch Hands had a lower adjusted mean count than the corresponding Comparisons, whereas the Ranch Hand current drinkers had higher adjusted mean counts than the corresponding Comparisons. The difference was large (68.9 cells/mm^3 versus 35.3 cells/mm^3) for the greater than four drinks per day category ($p=0.003$).

Significant effects on the monocyte counts were also seen for race ($p=0.032$), drink-years ($p=0.013$), current smoking ($p<0.001$), and pack-years ($p=0.006$). Analyses using Original Comparisons revealed a significant ($p=0.040$) group-by-age interaction (see Tables Q-6 and Q-7 of Appendix Q). This was due to a lower count for Ranch Hands than Comparisons for those born in or after 1942 (41.4 cells/mm^3 versus 48.0 cells/mm^3, $p=0.048$), a higher count for Ranch Hands than Comparisons for those born between 1923 and 1941 (48.2 cells/mm^3 versus 42.8 cells/mm^3, $p=0.058$), and very little difference for those born in or before 1922 ($p=0.924$).

HLA-DR Cells

No significant difference was found between groups in the mean values of HLA-DR cells ($p=0.842$). This contrast was analyzed without adjustment for any covariates except blood-draw day variation.

Significant associations were found using pooled data with occupation ($p<0.001$), age ($p=0.010$), current smoking ($p<0.001$), and pack-years ($p<0.001$). Officers had a lower mean count than enlisted participants (526 cells/mm^3 versus 597 cells/mm^3 for flyers and 598 cells/mm^3 for groundcrew). The average mean count was higher for younger participants than for

older participants: 588 cells/mm^3, 557 cells/mm^3, and 555 cells/mm^3 for those born in or after 1942, born between 1923 and 1941, and born in or before 1922, respectively. There was a significant increase in average mean counts with increasing levels of both current and lifetime smoking. There was a marginally significant increase in mean cell counts with drink-years ($p=0.083$).

Analyses, with adjustment for blood-draw day, each covariate, and any interactions, were carried out to assess the group difference in mean count of HLA-DR cells. A significant group-by-current alcohol use interaction was found ($p=0.045$); for Ranch Hands drinking more than four drinks per day, the adjusted mean count was greater, 564 cells/mm^3 versus 473 cells/mm^3, than for Comparisons ($p=0.052$), whereas no appreciable group differences were apparent for the participants drinking four or fewer drinks per day (see Table Q-1 of Appendix Q). Significant effects were seen with occupation ($p=0.035$), current smoking ($p<0.001$), and an age-by-pack-year interaction ($p=0.005$).

Analyses using Original Comparisons (Table Q-6 of Appendix Q) did not show a significant group-by-current alcohol use interaction ($p=0.152$), and no significant difference between groups was observed ($p=0.887$).

T_4/T_8 Ratio

No significant difference was found between groups in the mean value of the T_4/T_8 ratio ($p=0.499$). This contrast was analyzed without adjustment for any covariates except batch-to-batch variation.

The data were pooled for the two groups, and the relationship with the covariates was examined. Significant associations were found with drink-years ($p=0.049$), current smoking ($p<0.001$), and pack-years ($p=0.001$). The mean T_4/T_8 ratio generally increased with increasing drink-years, and increased with increasing amounts of current smoking and total pack-years. There was a marginally significant association with occupation ($p=0.063$). Enlisted flyers had a lower average ratio than officers and enlisted groundcrew (1.48 versus 1.62 and 1.60, respectively).

The adjusted group difference in the T_4/T_8 ratio was not significant ($p=0.678$; Ranch Hands 1.57 versus Comparisons 1.55). Significant effects were seen for occupation ($p=0.020$), current smoking ($p<0.001$), and batch-to-batch variation ($p<0.001$).

In the analysis of the Original Comparisons, a significant group-by-current smoking interaction was found ($p=0.016$). Further analysis showed a significant difference between groups (Ranch Hand mean ratio of 1.84 versus Original Comparison mean ratio of 1.51, $p=0.004$), for the greater than 20 to 40 cigarettes per day category (see Tables Q-6 and Q-7 of Appendix Q).

Functional Stimulation Studies

Statistical analyses were performed on cell function responses to PHA, PWM, and MLC. For each stimulated cell population, autologous controls were also studied. The measurements resulting from each test were the average counts over four samples for the stimulated cell population and for the autologous controls. The net average count, defined as the difference

between the average counts per minute (CPM) for the stimulated and the control cells, was also calculated.

Cell response data were obtained from the 1,085 immunologically tested participants. The exclusion conditions were the same as in the previous section, namely, participants who were taking anti-inflammatory or immunosuppressant medication, or who had recently experienced radiation therapy or chemotherapy for cancer.

Review of the immunological data base by the Air Force and SIRL resulted in certain test exclusions due to technical error, and equipment malfunction, those identified by quality control procedures (Grubbs' test[20]), and unexplained outliers. For the mean cell counts per minute (CPM) analyzed for this section, a total of 17 data points were excluded as unexplained outliers from the data base, involving eight participants (three Ranch Hands and five Comparisons): 1 point was invalid due to technical error in the assay for MLC stimulated cells (Ranch Hand) and the remainder were outliers in the PWM controls or stimulated cells (two Ranch Hand controls, 13 Comparison controls, and one Ranch Hand stimulated cells). This meant that, for one participant, the PWM control mean was omitted from the analysis and, for the other seven participants, the means were calculated from fewer than four points. No unexplained data points were found for the PHA-stimulated cells or corresponding controls.

All analyses were adjusted for significant blood-draw day variation, and the same covariates were used as in the adjusted analyses of the cell surface markers. The covariates age, current smoking, pack-years, current alcohol use, and drink-years were discretized because marginal examination showed generally nonlinear responses of the cell function variables with these covariates. Thus, the p-values given in this section for the marginal association of the variables with each covariate indicate the significance of the differences among the categories defined by the levels of the covariate.

Prior to analysis, the data were transformed to enhance normality or at least distributional symmetry. The following transformations were used:

Variable	Transformation
Unstimulated Response (PHA)	logarithm
PHA Net Response	none
Pokeweed Net Response	square root
MLC Net Response	square root

The summarized results of this section are given in Tables 19-6 through 19-8 (see Table Q-1 of Appendix Q for results involving group-by-covariate interactions). Only results for the unstimulated controls for the PHA assay are presented as an assessment of the function of the immune system in the unchallenged state. However, separate controls were run for each assay since incubation periods vary for each test procedure. In the analysis of data on the net response for each assay, the appropriate control was used. Analysis of each control assay was performed, and no significant group differences were noted.

TABLE 19-6.

Unadjusted Analyses for Functional Stimulation Tests by Group

Variable	Statistic*	Group: Ranch Hand	Group: Comparison	p-Value
Unstimulated Response (PHA)	n	464	584	
	Mean	1,656	1,657	0.979
	95% C.I.	(1,578, 1,737)	(1,589, 1,728)	
PHA Net Response	n	463	583	
	Mean	212,323	208,782	0.339
	95% C.I.	(206,484, 218,161)	(203,689, 213,875)	
Pokeweed Net Response	n	465	584	
	Mean	85,655	83,019	0.317
	95% C.I.	(81,528, 89,883)	(79,467, 86,648)	
MLC Net Response	n	452	564	
	Mean	79,132	82,460	0.185
	95% C.I.	(75,269, 83,092)	(79,010, 85,983)	

*Group means and confidence intervals expressed as counts per minute (CPM).

Unstimulated Response (PHA)

No significant difference was found between groups in the mean values of PHA unstimulated responses ($p=0.979$). These control values were derived from unstimulated cells and reflect baseline cell function. This contrast was analyzed without adjustment for any covariates except blood-draw day variation.

The data were pooled for the two groups, and the relationship with the covariates was examined. Significant associations were found with race ($p<0.001$), age ($p<0.001$), and drink-years ($p=0.048$). The average mean count for nonblacks was lower than for Blacks: 1,629 CPM and 2,210 CPM, respectively. There was a strong decrease in mean count with increasing age. For those born in or after 1942, the mean was 1,770 CPM; for those born between 1923 and 1941, the mean was 1,606 CPM; and for those born in or before 1922, the mean count was 1,238 CPM. The mean count generally decreased with increasing drink-years, with a maximum mean count of 1,726 CPM for non-drinkers and a minimum mean count of 1,414 CPM for participants with greater than 100 drink-years. A marginally significant association was found with occupation ($p=0.086$). The average mean count for officers was lower than that for enlisted flyers, which was in turn lower than that for enlisted ground-crew: the means were, respectively, 1,592 CPM, 1,662 CPM, and 1,713 CPM. This relationship with occupation was not seen with the PWM or MLC unstimulated responses. Since these values were derived from the same blood specimens and were unstimulated, this observation may represent a chance occurrence. There was a marginally significant association with pack-years ($p=0.051$); the response generally increased with increasing pack-years.

TABLE 19-7.

Association Between Functional Stimulation Test Variables and the Covariates in the Combined Ranch Hand and Comparison Groups (Directionality Shown)

Variable	Race	Occupation	Age	Current Alcohol (Drinks/Day)	Drink-Years	Current Smoking (Cigarettes/Day)	Lifetime Smoking Pack-years
Unstimulated Response (PHA)	<0.001 N<B	0.086 O<F<G	<0.001[a]	NS	0.048[b]	NS	0.051[c]
PHA Net Response	0.002 N<B	NS	<0.001[a]	<0.001[d]	0.002[d]	NS	NS
Pokeweed Net Response	NS	NS	NS	NS	0.038[e]	<0.001[f]	0.001[f]
MLC Net Response	NS	NS	0.035[b]	NS	0.008[g]	<0.001[f]	0.015[f]

[a]Monotone decreasing.
[b]Generally decreasing trend.
[c]Flat for 0 and first few categories, then increases.
[d]Increases, drop-off at highest category.
[e]Generally increasing trend.
[f]Monotone increasing.
[g]Increases from 0 category, then decreases, but not back to same level.

NS: Not significant (p>0.10).
N: Nonblack
B: Black
O: Officer
F: Enlisted flyer
G: Enlisted groundcrew

TABLE 19-8.

Adjusted Analyses for Functional Stimulation Tests by Group

Variable	Statistic*	Group: Ranch Hand	Group: Comparison	p-Value	Covariate: Remarks
Unstimulated Response (PHA)	n	464	584	0.855	BATCH (p<0.001)
	Mean	1,741	1,731		DAY (BATCH) (p<0.001)
	95% C.I.	(1,595, 1,901)	(1,593, 1,882)		RACE (p<0.001)
					AGE (p<0.001)
PHA Net Response	n	461	581	0.233	BATCH (p<0.001)
	Adj. Mean	193,280	188,952		DAY (BATCH) (p<0.001)
	95% C.I.	(176,032, 210,529)	(171,889, 206,014)		RACE (p=0.011)
					AGE*CSMOK (p=0.007)
					ALC*CSMOK (p=0.008)
Net Pokeweed Response	n	463	582	0.579	BATCH (p<0.001)
	Mean	91,567	90,097		DAY (BATCH) (p<0.001)
	95% C.I.	(82,189 101,451)	(81,008, 99,669)		RACE*OCC (p=0.024)
					ALC*OCC (p=0.036)
					ALC*CSMOK (p=0.009)
Net MLC Response	n	430	550	****	BATCH (p<0.001)
	Adj. Mean	****	****		DAY (BATCH) (p=0.001)
	95% C.I.	****	****		DRKYR (p<0.001)
					ALC (p=0.001)
					GRP*PACKYR (p=0.046)
					RACE*CSMOK (p=0.043)

*Group means and confidence intervals expressed as counts per minute (CPM).
****Group-by-covariate interaction--adjusted mean, confidence interval, and p-value not presented.

(The means were 1,657 CPM, 1,696 CPM, 1,519 CPM, and 1,712 CPM for 0, greater than 0 to 20, greater than 20 to 40, and greater than 40 pack-years, respectively.)

Adjusted analyses to assess the group difference in mean counts of PHA controls were performed with adjustment for each covariate and any interactions that made significant contributions, including significant blood-draw day variation. The group difference in adjusted mean count was not significant ($p=0.855$; Ranch Hand group mean of 1,741 CPM versus Comparison group mean of 1,731 CPM). Race and age were significant covariates ($p<0.001$ for both).

Adjusted and unadjusted analyses using the Original Comparisons (see Tables Q-8 and Q-9 of Appendix Q) showed similar results; i.e., no significant group difference ($p=0.608$, unadjusted; $p=0.613$, adjusted).

PHA Net Response

No significant difference was found between groups in the mean values of net response to PHA ($p=0.339$). This contrast was analyzed without adjustment for any covariates except blood-draw day variation.

The data were pooled for the two groups and the relationship with the covariates was examined. Significant associations were found for race ($p=0.002$), age ($p<0.001$), current alcohol use ($p<0.001$), and drink-years ($p=0.002$). Nonblacks had a lower net count than Blacks (208,953 CPM, 233,622 CPM, respectively). There was a steady decrease in net count with increasing age: the means were 217,003 CPM, 206,901 CPM, and 184,419 CPM for those born in or after 1942, born between 1923 and 1941, and born in or before 1922, respectively. Those currently drinking more than four drinks per day had a lower mean net count than those drinking less. The participants in the greater than 100 drink-year category had a lower mean net count than those with fewer drink-years.

Using a general linear model with adjustment for each covariate and any significant interactions including blood-draw day variation, the adjusted group difference was found to be not significant ($p=0.233$; Ranch Hand count of 193,280 CPM versus Comparison count of 188,952 CPM). Significant contributions were made by race ($p=0.011$), an age-by-current smoking interaction ($p=0.007$), and a current alcohol use-by-current smoking interaction ($p=0.008$).

A marginally significant ($p=0.057$) group-by-occupation interaction was excluded from the final model. However, this interaction was explored, and was found to be due to a group difference among enlisted flyers ($p=0.014$); the adjusted mean Ranch Hand net stimulated count was greater than that of the Comparisons (207,050 CPM and 185,344 CPM, respectively).

Analyses using the Original Comparisons (see Tables Q-9 and Q-10 of Appendix Q) revealed a significant group-by-occupation interaction ($p=0.017$), with results similar to the Ranch Hand versus total Comparison contrast of net counts; namely, enlisted flyer Ranch Hands had an adjusted mean count greater than enlisted flyer Original Comparisons ($p=0.003$).

Pokeweed Net Response

No significant difference was found between groups in the mean values of net response to pokeweed ($p=0.317$). These data were analyzed without adjustment for any covariates except blood-draw day variation.

Significant associations were found using the pooled group data with drink-years ($p=0.038$), current smoking ($p<0.001$), and pack-years ($p=0.001$). The mean count was higher for those with greater than 100 drink-years and lower for never-drinkers, but with no pattern for the in between categories. For both current and lifetime smoking (pack-years), there was a steady upward trend in mean counts with increasing levels of smoking.

The difference in adjusted group means was not significant: Ranch Hands, 91,567 CPM, and Comparisons, 90,097 CPM ($p=0.579$). The following interactions were significant: race-by-occupation ($p=0.024$), current alcohol use-by-occupation ($p=0.036$), and current alcohol use-by-current smoking ($p=0.009$).

Net Response to MLC Stimulation

No significant difference was found between groups in the mean response to MLC stimulation ($p=0.185$). These data were analyzed without adjustment for any covariates except blood-draw day variation.

The data were pooled for the two groups, and the relationship with the covariates was examined. Significant associations were found for age ($p=0.035$), drink-years ($p=0.008$), current smoking ($p<0.001$), and pack-years ($p=0.015$). The net mean count generally decreased with increasing age: 84,543 CPM, 72,408 CPM, and 79,081 CPM for those born in or after 1942, between 1923 and 1941, and in or before 1922, respectively. The net mean count was lowest for never-drinkers, with no clear pattern among the drinkers: 66,933 CPM, 78,555 CPM, 80,713 CPM, 84,236 CPM, and 80,416 CPM for the 0, greater than 0 to 5, greater than 5 to 30, greater than 30 to 100, and greater than 100 drink-year categories, respectively. There was a monotonically increasing trend in net average count with current smoking, the nonsmokers having a much lower value than the smokers. An equivalent pattern was found for lifetime smoking (pack-years).

Adjusted analyses were carried out to assess the group difference in mean counts of MLC net response, including adjustment for the significant blood-draw day variation. A significant group by pack-year interaction was found ($p=0.046$). Never-smoking Ranch Hands had a lower adjusted mean count (68,921 CPM) than the corresponding Comparisons (77,232 CPM) ($p=0.053$). Ranch Hands in the greater than 0 to 20 pack-year category had a lower adjusted mean count (67,976 CPM) than the corresponding Comparisons (74,333 CPM) ($p=0.057$). The adjusted means for the Comparisons decreased with increasing pack-years, whereas those of the Ranch Hands generally increased (see Table Q-1 of Appendix Q). Significant contributions were made to the model by drink-years ($p<0,001$), current alcohol use ($p=0.001$), and a race-by-current smoking interaction ($p=0.043$).

Discussion

The performance of the phenotypic and cell stimulation studies was monitored daily by highly structured quality assurance techniques (see Chapter 6). This resulted in a remarkably error-free data set, in contrast to the immunologic tests at the Baseline study that required the assistance of a review group to determine which data were appropriate for analysis. The finding of significant blood-draw day and batch-to-batch variation at the followup examination was judged to be totally normal and inherent within the test procedures; only a few data points within specific variables were omitted because of outlying values. The unique use of a "batch" variable for adjustment of all the phenotypic and stimulation studies permitted unadjusted and covariate-adjusted group contrasts while controlling for inherent laboratory variation.

All unadjusted and adjusted analyses (without group interactions) showed no significant group differences. Analysis of MLC revealed a group-by-pack-year of smoking interaction with lower counts in the Ranch Hand group than in the Comparison group for 0 and greater than 0 to 20 pack-year categories. Despite differences in the quality of Baseline and followup results, slight changes in cohort numbers, and different mathematical models, there was remarkable concordance in the immunologic results of both examinations, both for the dependent variables and for the effects of the covariates. No judgment of adverse immunologic competence was made for any variable, or sets of variables, or in substrata examined because of group-by-covariate interactions for the cell surface marker and cell stimulation studies.

EXPOSURE INDEX ANALYSES

Within each occupational category, exposure index analyses were conducted to assess possible dose-response relationships (see details in Chapter 8). Analyses were performed for the cell surface marker variables (total T cells, helper T cells, suppressor T cells, B cells, monocytes, HLA-DR cells, and the T_4/T_8 ratio) and for the functional stimulation tests (the control counts per minute for the PHA test, and the net PHA, PWM, and MLC counts per minute). Analyses were not done for the skin test responses.

Unadjusted and adjusted analyses were performed using general linear models. Exposure index-by-covariate interactions were explored in the adjusted analyses. Covariates were age, race, current and lifetime alcohol use (drink-years), and current and lifetime cigarette smoking (pack-years). For each analysis, an overall test was made of the differences among the means corresponding to the low, medium, and high exposure index levels. Medium versus low and high versus low contrasts of means were also made.

Results of the adjusted analyses are presented in Table 19-9 for cell surface markers and 19-10 for functional stimulation tests. Parallel results of unadjusted analyses are given in Tables Q-2 and Q-3, Appendix Q. Results of exposure index-by-covariate interactions are also given in Table Q-4 of Appendix Q.

TABLE 19-9.

Adjusted Exposure Index Analyses for Cell Surface Markers by Occupation

Variable	Occupation	Statistic	Exposure Index: Low	Medium	High	Contrast	p-Value
Total T Cells	Officer	n	62	62	55	Overall	0.672
		Adj. Mean	1,745	1,674	1,673	M vs. L	0.443
		95% C.I.	(1384,2148)	(1342,2042)	(1318,2069)	H vs. L	0.442
	Enlisted Flyer	n	21	24	24	Overall	0.874
		Adj. Mean	1,538	1,470	1,516	M vs. L	0.613
		95% C.I.	(1191,1928)	(1138,1845)	(1218,1847)	H vs. L	0.875
	Enlisted Groundcrew	n	65	76	53	Overall	0.068
		Adj. Mean	1,737	1,533	1,558	M vs. L	0.029
		95% C.I.	(1550,1935)	(1367,1709)	(1372,1756)	H vs. L	0.085
Helper T Cells	Officer	n	62	62	54	Overall	0.878
		Adj. Mean	798.2	801.1	774.3	M vs. L	0.960
		95% C.I.	(585.3,1044.1)	(600.8,1030.1)	(564.0,1071.9)	H vs. L	0.676
	Enlisted Flyer	n	21	25	23	Overall	0.726
		Adj. Mean	824.1	760.0	788.4	M vs. L	0.426
		95% C.I.	(612.8,1066.7)	(561.6,988.4)	(609.2,990.7)	H vs. L	0.672
	Enlisted Groundcrew	n	65	74	53	Overall	0.150
		Adj. Mean	968.6	874.2	867.7	M vs. L	0.090
		95% C.I.	(858.0,1086.0)	(774.4,980.1)	(758.1,984.7)	H vs. L	0.101

TABLE 19-9. (continued)

Adjusted Exposure Index Analyses for Cell Surface Markers by Occupation

Variable	Occupation	Statistic	Exposure Index: Low	Medium	High	Contrast	p-Value
Suppressor T Cells	Officer	n	62	63	55	Overall	0.269
		Adj. Mean	648.1	566.9	606.3	M vs. L	0.106
		95% C.I.	(463.9,905.4)	(414.7,774.9)	(433.4,848.1)	H vs. L	0.425
	Enlisted Flyer	n	21	25	24	Overall	0.930
		Adj. Mean	500.5	493.5	518.8	M vs. L	0.915
		95% C.I.	(347.5,720.8)	(345.1,705.5)	(379.0,710.1)	H vs. L	0.792
	Enlisted Groundcrew	n	65	75	53	Overall	0.088
		Adj. Mean	558.6	480.8	483.7	M vs. L	0.044
		95% C.I.	(481.4,648.2)	(417.6,553.6)	(413.6,565.5)	H vs. L	0.081
B Cells	Officer	n	62	63	54	Overall	0.857
		Adj. Mean	141.2	137.7	147.0	M vs. L	0.828
		95% C.I.	(82.2,216.2)	(82.7,206.5)	(86.3,223.8)	H vs. L	0.735
	Enlisted Flyer	n	21	24	22	Overall	0.283
		Adj. Mean	229.0	202.6	174.2	M vs. L	0.438
		95% C.I.	(143.0,335.2)	(123.4,301.3)	(109.5,253.9)	H vs. L	0.114
	Enlisted Groundcrew	n	65	71	53	Overall	0.900
		Adj. Mean	215.9	206.4	209.9	M vs. L	0.650
		95% C.I.	(176.2,259.6)	(169.3,247.3)	(168.9,255.4)	H vs. L	0.794

TABLE 19-9. (continued)

Adjusted Exposure Index Analyses for Cell Surface Markers by Occupation

Variable	Occupation	Statistic	Exposure Index Low	Medium	High	Contrast	p-Value
Monocytes	Officer	n	62	63	54	Overall	0.988
		Adj. Mean	45.70	46.75	46.31	M vs. L	0.878
		95% C.I.	(24.95,83.69)	(26.56,82.31)	(25.21,85.06)	H vs. L	0.930
	Enlisted Flyer	n	21	25	23	Overall	0.605
		Adj. Mean	25.81	26.49	31.68	M vs. L	0.903
		95% C.I.	(14.30,46.57)	(14.86,47.21)	(19.07,52.61)	H vs. L	0.356
	Enlisted Groundcrew	n	65	74	53	Overall	0.836
		Adj. Mean	43.00	41.64	44.84	M vs. L	0.778
		95% C.I.	(34.22,54.03)	(33.52,51.72)	(35.28,54.00)	H vs. L	0.739
HLA-DR Cells	Officer	n	62	62	55	Overall	0.963
		Adj. Mean	552.6	563.9	564.9	M vs. L	0.819
		95% C.I.	(370.7,770.6)	(390.9,768.6)	(380.2,786.0)	H vs. L	0.807
	Enlisted Flyer	n	21	25	24	Overall	0.912
		Adj. Mean	501.9	479.5	504.8	M vs. L	0.729
		95% C.I.	(337.6,698.7)	(322.1,668.1)	(360.9,672.7)	H vs. L	0.966
	Enlisted Groundcrew	n	65	74	52	Overall	0.903
		Adj. Mean	591.0	584.4	571.9	M vs. L	0.864
		95% C.I.	(515.5,672.1)	(512.6,660.9)	(492.1,657.8)	H vs. L	0.654

TABLE 19-9. (continued)

Adjusted Exposure Index Analyses for Cell Surface Markers by Occupation

Variable	Occupation	Statistic	Exposure Index: Low	Exposure Index: Medium	Exposure Index: High	Contrast	p-Value
T_4/T_8 Ratio	Officer	n	62	62	54	Overall	0.204
		Adj. Mean	1.326	1.575	1.285	M vs. L	0.152
		95% C.I.	(0.626,2.026)	(0.921,2.229)	(0.585,1.985)	H vs. L	0.816
	Enlisted Flyer	n	21	25	23	Overall	0.788
		Adj. Mean	1.675	1.539	1.552	M vs. L	0.527
		95% C.I.	(1.079,2.271)	(0.955,2.122)	(1.040,2.064)	H vs. L	0.583
	Enlisted Groundcrew	n	65	74	53	Overall	0.909
		Adj. Mean	1.833	1.878	1.827	M vs. L	0.716
		95% C.I.	(1.587,2.079)	(1.645,2.112)	(1.569,2.086)	H vs. L	0.965

TABLE 19-10.

Adjusted Exposure Index Analyses for Functional Stimulation Tests by Occupation

Variable	Occupation	Statistic	Exposure Index Low	Medium	High	Contrast	p-Value
Unstimulated Response (PHA)	Officer	n	****	****	****	Overall	****[a]
		Adj. Mean	****[a]	****[a]	****[a]	M vs. L	****[a]
		95% C.I.	****[a]	****[a]	****[a]	H vs. L	****[a]
	Enlisted Flyer	n	21	25	24	Overall	0.731
		Adj. Mean	2,162	2,489	2,167	M vs. L	0.501
		95% C.I.	(941,4966)	(1195,5185)	(1088,4659)	H vs. L	0.991
	Enlisted Groundcrew	n	65	76	52	Overall	0.713
		Adj. Mean	1,893	2,000	2,067	M vs. L	0.579
		95% C.I.	(1366,2624)	(1433,2793)	(1481,2886)	H vs. L	0.427
PHA Net Response	Officer	n	****	****	****	Overall	****[a]
		Adj. Mean	****[a]	****[a]	****[a]	M vs. L	****[a]
		95% C.I.	****[a]	****[a]	****[a]	H vs. L	****[a]
	Enlisted Flyer	n	21	25	24	Overall	0.456
		Adj. Mean	276,274	240,021	251,070	M vs. L	0.222
		95% C.I.	(158,921, 393,628)	(136,445, 343,597)	(143,073, 359,068)	H vs. L	0.382
	Enlisted Groundcrew	n	65	76	52	Overall	0.482
		Adj. Mean	222,416	220,417	204,601	M vs. L	0.888
		95% C.I.	(175,692, 269,141)	(172,635, 268,198)	(156,815, 252,386)	H vs. L	0.261

TABLE 19-10. (continued)

Adjusted Exposure Index Analyses for Functional Stimulation Tests by Occupation

Variable	Occupation	Statistic	Exposure Index Low	Medium	High	Contrast	p-Value
Pokeweed Net Response	Officer	n	62	63	55	Overall	0.674
		Adj. Mean	123,706	118,404	131,711	M vs. L	0.711
		95% C.I.	(69,007, 194,255)	(67,130, 184,129)	(74,319, 205,414)	H vs. L	0.596
	Enlisted Flyer	n	21	25	24	Overall	0.025
		Adj. Mean	173,897	164,642	111,772	M vs. L	0.737
		95% C.I.	(80,411, 302,996)	(89,936 274,089)	(44,741, 208,963)	H vs. L	0.014
	Enlisted Groundcrew	n	65	76	52	Overall	0.392
		Adj. Mean	105,624	121,674	110,807	M vs. L	0.180
		95% C.I.	(71,257, 146,732)	(83,779, 166,618)	(74,803, 153,863)	H vs. L	0.689
MLC Net Response	Officer	n	58	62	53	Overall	0.977
		Adj. Mean	95,550	98,079	97,973	M vs. L	0.847
		95% C.I.	(47,962, 159,377)	(51,645, 159,278)	(49,028, 163,692)	H vs. L	0.859
	Enlisted Flyer	n	20	25	22	Overall	0.491
		Adj. Mean	114,713	93,189	86,844	M vs. L	0.387
		95% C.I.	(35,112, 240,104)	(29,607, 192,257)	(24,482, 187,417)	H vs. L	0.249
	Enlisted Groundcrew	n	64	75	51	Overall	0.251
		Mean	61,403	78,259	69,881	M vs. L	0.097
		95% C.I.	(34,314, 96,320)	(46,477, 118,275)	(40,193, 107,728)	H vs. L	0.437

[a]Group-by-covariate interaction--adjusted mean, confidence interval, and p-value not presented.

Cell Surface Markers

Unadjusted analyses revealed very few significant results. Among enlisted groundcrew, the medium exposure level had a significantly lower mean total T cell count than the low exposure level (1,555 cells/mm^3 versus 1,759 cells/mm^3, p=0.032), and the high exposure level mean was marginally significantly (p=0.091) lower than the low exposure level mean (1,586 cells/mm^3 versus 1,759 cells/mm^3). Suppressor T cells, for enlisted groundcrew in the low exposure level, were marginally significantly higher than in the medium or high exposure levels (575.5, 502.3, 505.5 cells/mm^3, respectively: medium versus low, p=0.063, high versus low, p=0.097). For enlisted flyers, the trends with exposure level were steadily downwards for total T cells, helper T cells, B cells, and the T_4/T_8 ratio, and upwards for suppressor T cells and monocytes, but no contrasts were significant.

Adjusted analyses revealed marginally significant differences among exposure levels of enlisted groundcrew for total T cells (p=0.068) and suppressor T cells (p=0.088). For both total and suppressor T cells, the means for the medium and high exposure levels were much lower than those of the low exposure level. For total T cells, the adjusted means were: low, 1,737 cells/mm^3; medium, 1,533 cells/mm^3; and high, 1,558 cells/mm^3 (medium versus low: p=0.029, high versus low: p=0.085). For suppressor T cells, the adjusted means were: low, 558.6 cells/mm^3; medium, 480.8 cells/mm^3; and high, 483.7 cells/mm^3 (medium versus low p=0.044, high versus low p=0.081). A similar but less marked pattern was seen for helper T cells.

In summary, there was no consistent evidence of any significant dose-response pattern in an occupational category. For the enlisted flyer cohort, six of the seven variables revealed nonsignificant dose-response trends in the unadjusted analyses, but only two trends persisted after adjustment by the covariates.

Functional Stimulation Tests

Exposure index analyses were performed on PHA unstimulated responses, and net PHA, PWM, and MLC counts. For officers, the unadjusted mean PHA unstimulated response counts varied significantly among exposure levels (p=0.047). The means were 1,705 CPM, 1,428 CPM, and 1,809 CPM, respectively, for low, medium, and high exposure levels (medium versus low: p=0.071, high versus low: p=0.557). The PWM net count for enlisted flyers was significantly lower for the high versus the low exposure levels (55,480 CPM versus 92,847 CPM, p=0.011). The PHA net count had a downward trend with increasing exposure level for enlisted groundcrew. The PWM net count for officers had an increasing trend but there was no statistically significant difference among exposure levels.

In the adjusted analyses, officers had a significant exposure index-by-drink-year interaction (p=0.011) for PHA controls, and an exposure index-by-age interaction for the PHA net count (p=0.003) (see Table 19-11 for a summary of these interactions). Although the numbers were small, the high

TABLE 19-11.

Summary of Exposure Index by Covariate Interactions for Functional Stimulation Tests

Variable	Occupation	Covariate	p-Value
Unstimulated Response (PHA)	Officer	Drink-years	0.011
PHA Net Response	Officer	Age	0.003

exposure-level nondrinking officers had a lower mean PHA control count than the low exposure level (1,557 CPM versus 3,273 CPM, p=0.031), and the high exposure level officers with more than 100 drink-years had a higher PHA control count than the corresponding low exposure group (6,700 CPM versus 1,983 CPM, p=0.049). Officers born in or after 1942 had a lower PHA net count in the medium exposure level as contrasted with the low exposure level (153,534 CPM versus 261,397 CPM, p=0.002).

Other adjusted analyses revealed that enlisted flyers had a lower PWM net count in the high exposure level as compared to the low exposure level (111,772 CPM versus 173,897 CPM, p=0.014), as in the unadjusted analysis. Enlisted groundcrew in the medium exposure level also had a marginally significantly higher MLC net count as compared to the low exposure level (78,259 CPM versus 61,403 CPM, p=0.097).

In summary, there was no evidence for a strong dose-response relationship, but there was a trend for declining PWM and MLC net counts for enlisted flyers with increasing exposure level.

SKIN TESTING RESULTS

General

Four skin test antigens, mumps, Candida albicans, Trichophyton, and staph-phage-lysate, were intradermally administered to 76 percent (1,759) of the participants on the first day of the examination. Skin tests were not given to the remaining 24 percent of the population because they had been selected to give blood for the immunological tests on the second day of their examination. Candida albicans and Trichophyton tests were administered (0.1 ml) at a 1:1000 weight/volume dilution because of clinical concern that a 1:100 or higher concentration might induce significant skin reactions and cause morbidity in the active pilot population. Mumps was given at a dose of 2 complement-fixing units, and staph-phage-lysate was administered at a dose of 6-9 x 10^6 colony-forming units of Staph. aureus and 0.5 - 5 x 10^8 bacteriaphage plaque-forming units.

Three experienced technicians from the SCRF Allergy Division measured the size of both induration and skin erythema by the "pen method" at 24 and 48 hours after administration. Each reader was required to measure the skin reactions by a millimeter ruler and record length and breadth measurements at each of the four sites, refer exaggerated reactions to an allergist, collect medication use data, and sign the data form. The skin test data were interpreted by defined criteria, as given in Table 19-12. Other categories included: impairment noted, clinical correlation required; normal (versus abnormal) results; with medications noted; and refusal.

Of the 1,759 participants with skin tests, 269 were excluded from the analyses for the following reasons: 205 due to missing reader signature or failure of the participant to report for the 48-hour reading; 58 because of immunosuppressive medication, cancer chemotherapy, or x-ray therapy; 3 for impaired hypersensitivity requiring more tests; and 3 due to refusal. Readings at 24 hours were not analyzed since these readings occurred prior to peak response to the antigens.

Statistical Analyses and Interpretations

The initial analytical intent was to test Ranch Hand-Comparison group differences in skin test response by standard models, using both discrete and continuous data. In the preanalysis of the continuously distributed data (length by width measurement of the skin reactions), there was a suggestion of profound reader variation. This observation generated a series of contrasts between the readers prior to group testing.

Figures 19-1 through 19-6 show contrasts of the three skin test readers from the tests of mumps and Trichophyton. Each graph shows the individual plots of the 48-hour induration square area measurement versus the 48-hour

TABLE 19-12.

Clinical Interpretation Categories of Skin Test Results by Specific Measurement Criteria at SCRF

Clinical Interpretation Category	Measurement Criteria
Normal (Delayed Cutaneous Hypersensitivity Intact)	Length (L) induration @ 48 Hrs ≥10 mm Width (W) induration @ 48 Hrs ≥ 10 mm on <u>any one</u> of four skin tests
Probably Normal (Probably Intact Delayed Cutaneous Hypersensitivity)	L induration @ 48 Hrs ≥ 5-<10 mm W induration @ 48 Hrs ≥ 5-<10 mm on any one of four skin tests
Possibly Anergic	L, W induration <u>or</u> erythema @ 48 Hrs >0-<5 mm on any one or more of four skin tests
Anergic	L and W, induration at 48 hrs = 0 on all skin tests.

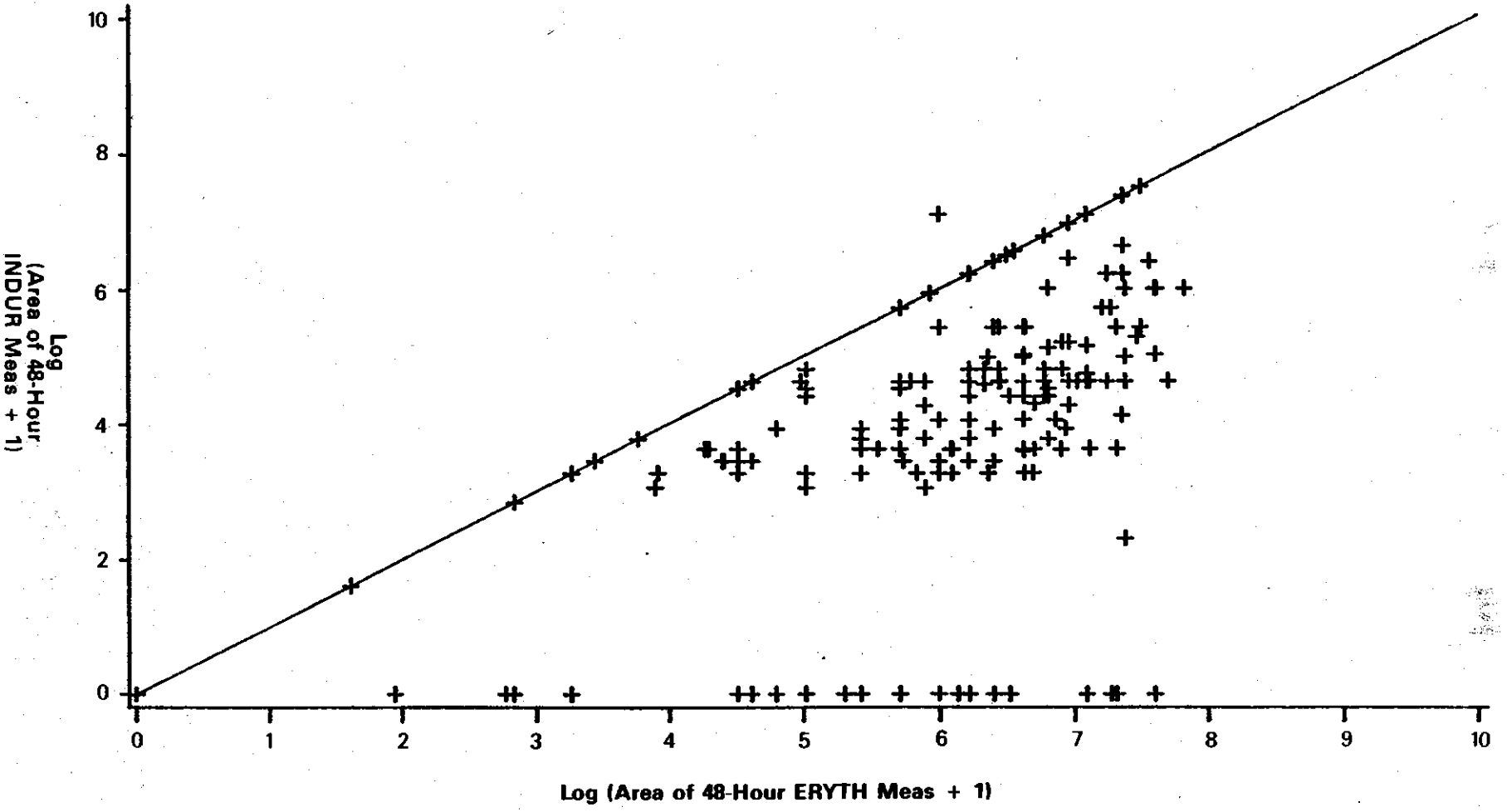

Figure 19-1.
Relationship of Induration Measurements to Erythema Measurements for the Mumps Skin Test
Reader 1 Results

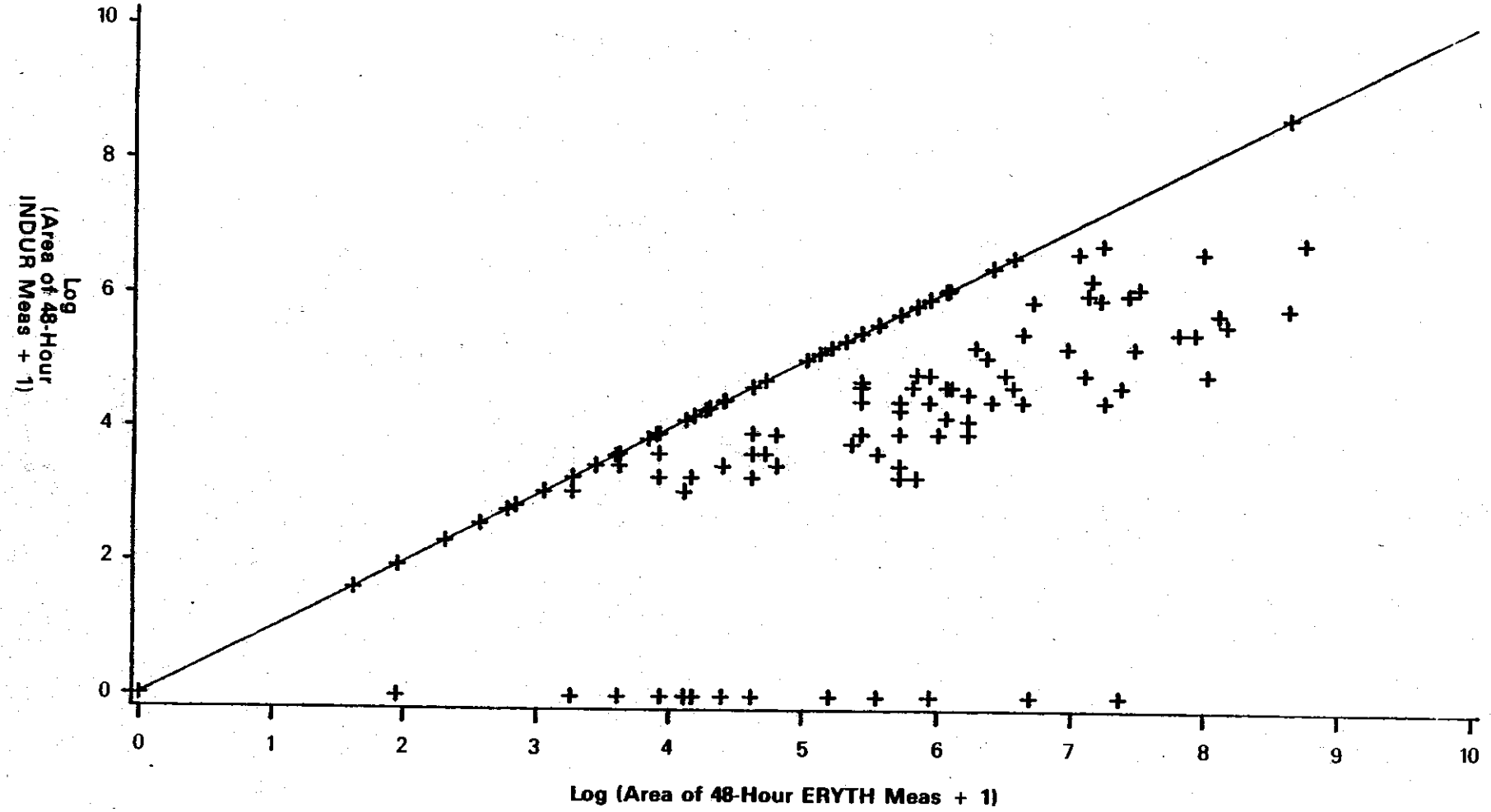

Figure 19-2.
Relationship of Induration Measurements to Erythema Measurements for the Trichophyton Skin Test.
Reader 1 Results

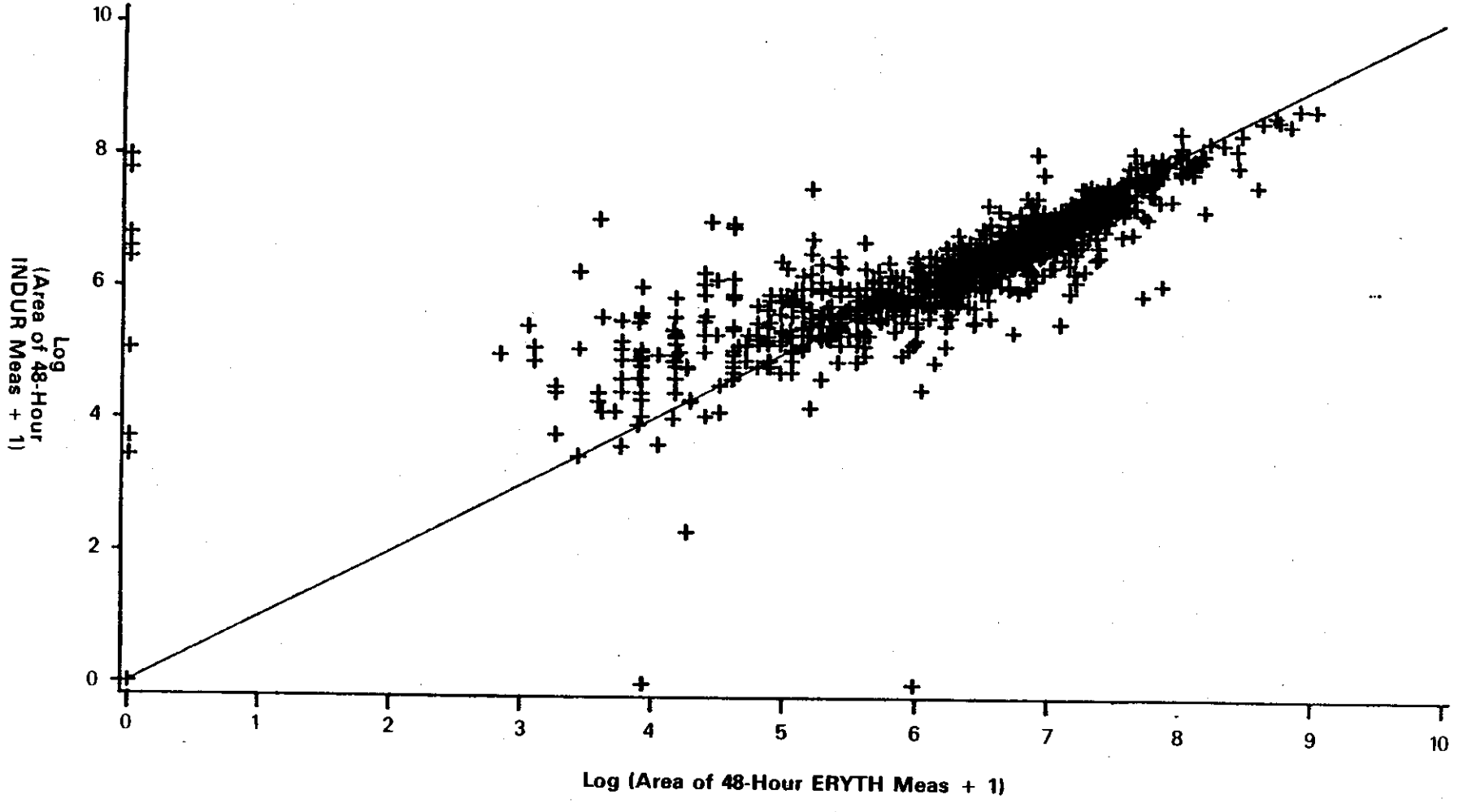

Figure 19-3.
Relationship of Induration Measurements to Erythema Measurements for the Mumps Skin Test
Reader 2 Results

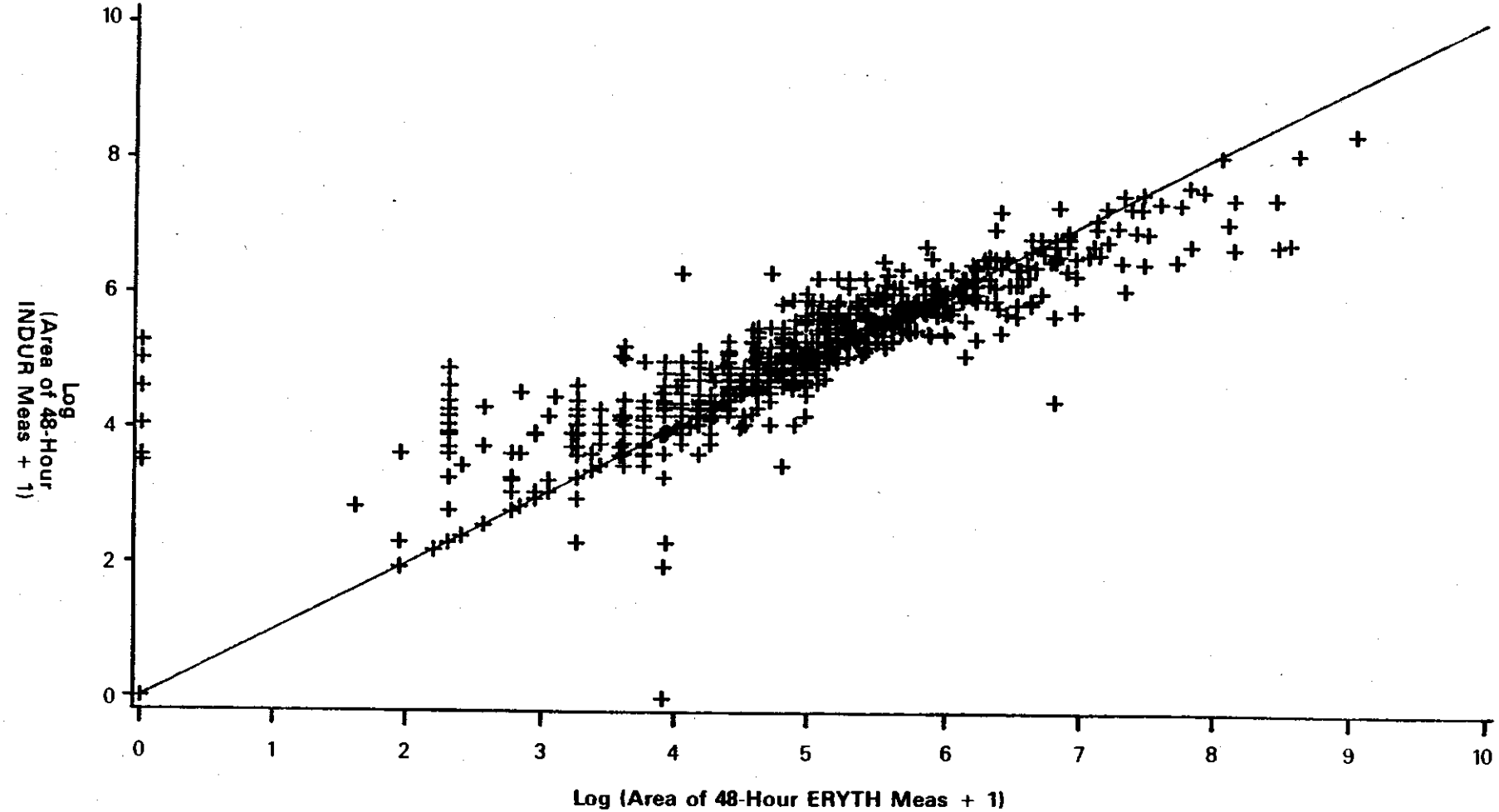

Figure 19-4.
Relationship of Induration Measurements to Erythema Measurements for the Trichophyton Skin Test
Reader 2 Results

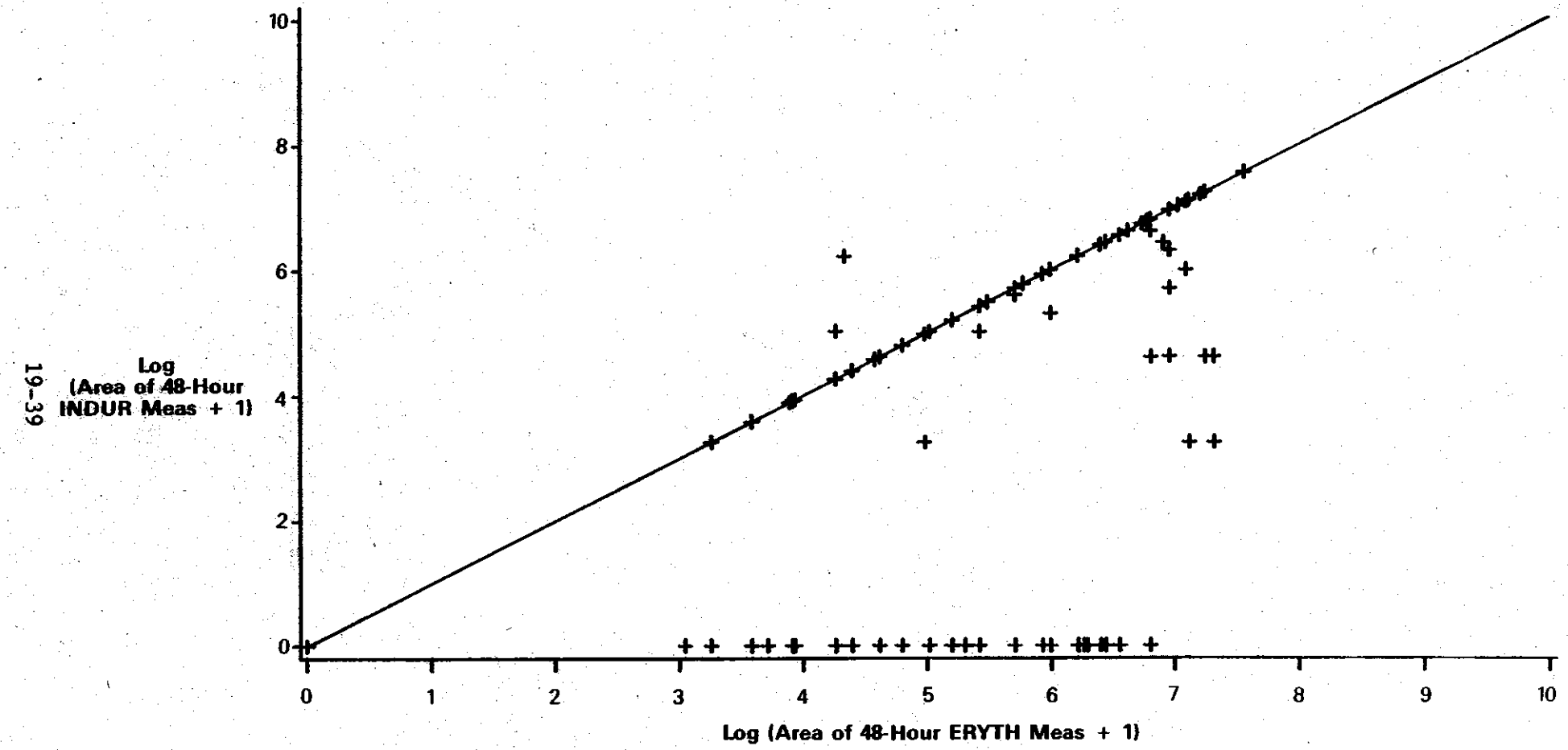

Figure 19-5.
Relationship of Induration Measurements to Erythema Measurements for the Mumps Skin Test
Reader 3 Results

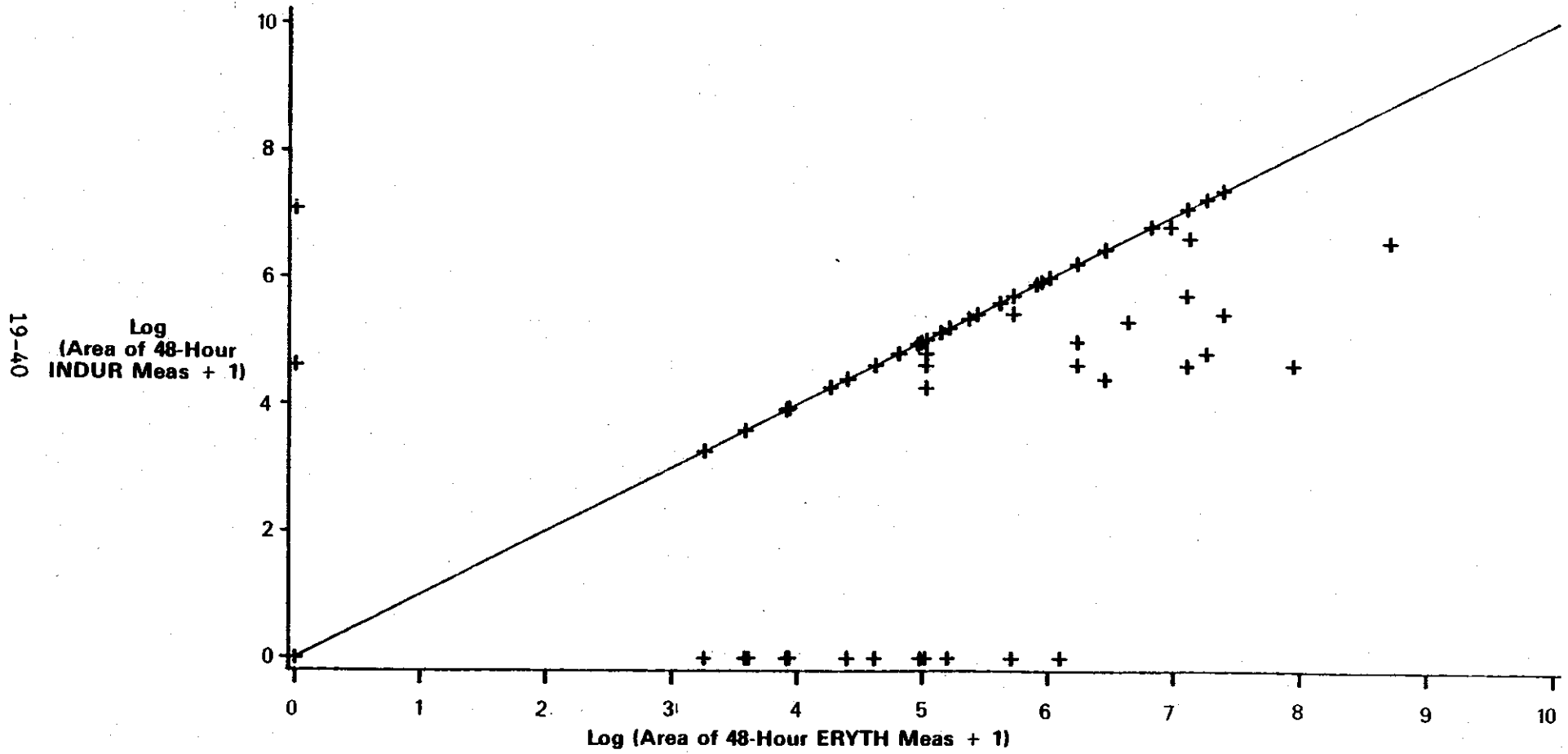

Figure 19-6.
Relationship of Induration Measurements to Erythema Measurements for the Trichophyton Skin Test
Reader 3 Results

square area erythema measurement by specific skin test and reader. These measurements are presented in log units to centralize the outlying values. These analyses were done because the size of induration rarely exceeds the size of the erythema reaction. Thus, each of the depicted graphs shows a line of values with the sizes of erythema equal to the size of induration; this line, and all values on, or to the lower right of the line, are labeled "clinically acceptable" values. All values above and to the left of the line, deemed "clinically unacceptable," are probably due to hurried measurements by inspection (rather than the pen method) or recording errors.

These figures demonstrated a marked difference in the occurrence of clinically unacceptable results between readers for comparable tests. Specifically, Reader 2's measurements revealed a higher proportion of clinically unacceptable results than those observed with Readers 1 and 3. Further, the graphs supported some variation in the clinically acceptable measurement values between Reader 1 and Reader 3. Because of these discordances, further analyses of the continuously distributed data were abandoned in favor of discretized analyses.

Categorical analyses were conducted on two parameters of the skin testing results, the area measurement relationship of induration to erythema, and the clinical interpretation of the skin test readings. Each of the three readers was compared for 48-hour measurements on the same skin test, categorizing the induration-erythema relationship as (1) Induration (I) equals Erythema (E) (both values equal to zero), (2) E greater than I, (3) I equals E, and (4) I greater than E. As previously noted, only the category of I greater than E was judged clinically unacceptable. An analysis of these four categories, by reader, for each of the four skin tests, showed a profound statistical difference ($p<0.001$) between the readers for all four skin tests. An average of the percentages for each category by reader is shown in Table 19-13, exemplifying the marked differences (a p-value is inappropriate due to the averaging).

TABLE 19-13.

Induration Erythema Relationships in Average Percentage Over Four Skin Tests, by Reader

In Percent

Reader	I=E=Zero*	E>I	I=E	I>E
1	26.2	53.9	19.8	0.1
2	7.0	42.3	20.7	30.0
3	24.1	35.4	39.6	0.9

*I:Induration
E:Erythema

These data show marked reader differences for the category I greater than E. The magnitude of clinically unacceptable results (30.0% on the average for four skin tests) for Reader 2 (visually shown in Figures 19-3 and 19-4) strongly suggested that this entire data set was invalid. Further, the data pattern from Reader 2 was shown to be uniform over time, confirming the existence of a consistent bias. In this light, the existence and magnitude of a reverse error for Reader 2, i.e., misreadings of I equals E equals 0, E greater than I, and I equals E, seem plausible, but unestimable. Of these three categories, I equals E equals 0 is the most clinically important (suggesting anergy), and Table 19-13 provides clear evidence of a negative bias, with Readers 1 and 3 showing over three times more average detection of anergy than Reader 2. Also of interest in Table 19-13 are the substantial differences in the categories E greater than I and I equals E for Readers 1 and 3. Analyses of the four skin tests by erythema-induration relationships showed statistically significant differences beween Readers 1 and 3 for all four tests ($p<0.001$ for mumps, Candida albicans, and Trichophyton, and $p=0.036$ for staph-phage-lysate).

The decision to remove Reader 2 data from subsequent analysis was agreed to by all the Principal Investigators, recognizing the minimal role of erythema as a contemporary indicator of anergy. This decision was based on the concern that an error in erythema measurement likely indicated an error in measurement of induration (the predominant indicator of anergy).

In preparation for the analysis of group data remaining from Readers 1 and 3, it was noted that the clinical interpretations (see Table 19-12) from these valid readings were inconsistent over time of the study. Specifically, 80 percent of relative anergy and anergy occurred in the first 10 of 81 groups of participants (or 2 1/2 months of the 9-month examination period). Further, the proportion of diagnoses of anergy between the allergists was disproportionate. The value of these analyses was therefore reduced.

SUMMARY AND CONCLUSIONS

Immunologic competence was measured by cell surface marker (phenotypic) studies and cell stimulation studies on 47 percent of the study population, and by a four antigen series of skin tests in 76 percent of participants to assess the delayed hypersensitivity response. Table 19-14 summarizes the results of all unadjusted and adjusted analyses on 11 primary variables spanning the first two of these three functional areas.

Cell surface marker studies were conducted for total T cells (T_{11}), helper T cells (T_4), suppressor T cells (T_8), B cells, monocytes, and HLA-DR cells; the ratio of T_4/T_8 cells was included in the analysis. Because of inherent significant day-to-day and batch-to-batch variation, all results (including functional stimulation studies) were adjusted for blood-draw day variation. Statistical testing of the seven phenotypic cell markers did not reveal any significant group differences (interactions excepted), either unadjusted or adjusted for the covariates of age, race, occupation, current smoking, lifetime smoking history (pack-years), current alcohol use, or lifetime alcohol use (drink-years). Similarly, none of the unadjusted or adjusted analyses of the functional stimulation studies (for phyto-hemagglutinin, pokeweed mitogen, or mixed lymphocyte culture) showed any

TABLE 19-14.

Overall Summary Results
of Unadjusted and Adjusted
Analyses of Immunological Variables

Variable	Unadjusted	Adjusted
Total T Cells (T_{11})	NS	****
Helper T Cells (T_4)	NS	NS
Suppressor T Cells (T_8)	NS	NS
B Cells	NS	****
Monocytes	NS	****
HLA-DR Cells	NS	****
T_4/T_8 Ratio	NS	NS
Unstimulated Response (PHA)	NS	NS
PHA Net Response	NS	NS
Pokeweed Net Response	NS	NS
MLC Net Response	NS	****

NS:Not significant (p>0.10).
****Significant group-by-covariate interaction.

statistically significant group differences. However, the adjusted analyses for total T cells, B cells, monocytes, HLA-DR cells, pokeweed mitogen, and net mixed lymphocyte culture stimulation showed some significant group-by-covariate interactions, precluding direct adjusted group contrasts. Overall, no discernible pattern was identified to suggest a detriment in any subgroup of either the Ranch Hands or Comparisons. Results were similar between the analyses of the total Comparison group and the analyses of the Original Comparisons.

The covariate effects of age, race, smoking, and alcohol use were generally profound on most variables in the phenotypic and stimulation studies. Consistently decreasing values of all cell markers and stimulated cells were associated with increasing age, whereas increased levels of smoking were usually associated with increases in the values of those variables. Blacks had consistently higher stimulated cell counts than nonblacks, but this effect was not observed for counts of T cells, B cells, or HLA-DR cells. Enlisted personnel generally had higher cell surface marker counts than officers.

Exposure index analyses of cell surface markers revealed no pattern consistent with a dose-response relationship. For enlisted groundcrew, the mean total T cell and suppressor T cell counts for the medium exposure level were significantly lower than those of the low exposure level, but were slightly lower than those of the high exposure level. The exposure index analyses of the functional stimulation tests revealed no consistent significant dose-response patterns for net PHA counts or net MLC counts. For net pokeweed counts, enlisted flyers in the high exposure level had a significantly lower adjusted count than enlisted flyers in the low exposure level, and a decreasing trend was apparent.

The delayed hypersensitivity response was assessed by the skin test antigens of mumps, Candida albicans, Trichophyton, and staph-phage-lysate. The 48-hour measurements of skin induration and erythema for the four tests showed marked inter-reader variation. Analyses showed that one of the three skin test readers too often measured induration larger than erythema (a clinically unacceptable finding), in an average of 30 percent of the readings, and did not yield measurements that detected a case of possible or overt anergy, whereas the other two readers found this condition in 5.6 percent of the participants. Remaining data from Readers 1 and 3, however, were found to vary significantly in clinical interpretation over duration of the examination. Consequently, all skin test data were declared invalid, and were not used in the assessment of group differences. The skin test reading problems led to the use of additional clinical quality control procedures for the AFHS followup examination begun in May 1987.

In conclusion, no significant group differences were judged present for the comprehensive cell surface marker or functional stimulation studies. The profound effects of age, smoking, and alcohol use were observed in these immunologic tests. The assessment of delayed hypersensitivity skin responses was precluded by poor data quality and excluded from further analysis. Overall, there was no indication of impaired immunologic competence in either group.

CHAPTER 19

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CHAPTER 20

PULMONARY DISEASE

INTRODUCTION

Pulmonary dysfunction and overt pulmonary disease are not recognized clinical entities resulting from exposure to chlorophenols or TCDD.

Acute exposure to chlorophenols, phenoxy herbicides, and TCDD, have caused the traditional acute symptoms of cough, nasal/lung irritation, shortness of breath, and, occasionally, bronchitis. These symptoms have been noted almost exclusively in industrial workers and not in individuals experiencing casual contact. Long-term sequelae arising from the acute symptom stage in ill individuals have not been generally known because of minimal followup and surveillance of the pulmonary symptoms.

Only one contemporary morbidity study has attributed pulmonary dysfunction to phenoxy herbicide and TCDD exposure.[1] The percent abnormal pulmonary parameters of forced expiratory volume (FEV), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1)/FVC ratio, and forced midexpiratory flow rate (FEF_{25-75}) were significantly higher in exposed workers who currently smoke, than in nonexposed workers who smoke. In considerable contrast, these test parameters were essentially equal in nonsmokers and former smokers of both the exposed and nonexposed groups. The effect of current smoking persisted after a logistic regression analysis adjusting for pack-years of cigarette smoking. Adjusted means of the test parameters FEV, FVC, and FEV_1/FVC also showed significant differences for current smokers but not for nonsmokers or former smokers.

As with other nonclassical clinical endpoints, prior investigators perhaps undervalued the incorporation of pulmonary disease and function into their study protocols.

Further, due to the profound effect of smoking on pulmonary function, great emphasis must be placed in the collection of highly accurate, detailed, and validated smoking data as an adjustment variable, a process that is not straightforward in today's environment of antismoking.

The only recent data comparable to this study are found in the 1984 AFHS Baseline Morbidity Report, which is reviewed below.[2]

Baseline Summary Results

The 1982 Baseline examination explored historical pulmonary disease by questionnaire and active pulmonary function by standardized spirometric technique at the physical examination. These areas were of significant interest because of routine operational inhalation of Herbicide Orange by all Ranch Hand flying crewmen as well as ground maintenance personnel (Baseline Report Chapter 1, Buckingham).

The questionnaire revealed no group differences for historical diagnoses of tuberculosis and fungal infections, pneumonia, cancer, or chronic sinusitis and upper respiratory disease. At the physical examination the unadjusted means for FEV_1 (percent predicted), FVC, and the FEV_1/FVC ratio were almost identical between the Ranch Hands and Comparisons. Adjusted mean values were not calculated due to significant interactions (age, group, and pulmonary function for FEV_1 and FVC; smoking with FEV_1/FVC).

Detailed exposure analyses showed two significant associations in the enlisted flyer and enlisted groundcrew strata, but neither was indicative of linear dose response. Attempts to adjust the means of the pulmonary function values for age and smoking revealed several interactions, but essentially negative results.

Overall, there were no pulmonary disease or pulmonary function data or associations of concern.

Parameters of the 1985 Pulmonary Examination

Because of the essentially negative pulmonary analyses from the Baseline examination, pulmonary function (spirometric) studies were not performed during the first followup examination. Collection of pulmonary data was limited to a questionnaire history of respiratory disease, physical examination of the thorax and lungs, and pulmonary abnormalities detected on a routine chest x ray.

Thus, the data analyses consist of group assessments of respiratory disease incidence, physical examination abnormalities, and the current prevalence of x-ray abnormalities. Covariate adjustments are made for age and smoking (yes, no, former, and pack-years). Minor numeric differences in the tables are due to rare missing dependent variable or covariable data. The analyses are based on 1,016 Ranch Hands and 1,293 Comparisons. No exclusions based on clinical conditions were made.

Mortality due to respiratory disease, as of 31 December 1985, in the Ranch Hand and the 1:5 matched Comparison cohort is summarized. Morbidity data are analyzed using linear and loglinear models.

RESULTS AND DISCUSSION

Mortality Experience

The mortality of the Ranch Hand and Comparison groups through 31 December 1985 was evaluated. There were seven deaths from respiratory system conditions in the Comparison group and none in the Ranch Hand group. This analysis was based on the 1:5 Ranch Hand to Comparison mortality study cohorts. Two of these deaths were Comparison flying officers, three were enlisted flyers, and the remaining two were enlisted groundcrew.

Unadjusted Morbidity Analyses

Analyses were performed on the history of respiratory illnesses as provided by the participants during the physical examination. The results of the

radiological and clinical examination of the lungs and chest were also analyzed. These unadjusted analyses are summarized in Tables 20-1 and 20-2.

As shown, no significant group differences were observed for history of asthma, bronchitis, pleurisy, pneumonia, or tuberculosis. Similar non-significant results were found in the evaluation of the clinical variables.

Parallel analyses were conducted using data from the Original Comparisons, with comparable results (Appendix R, Table R-1).

Adjusted Morbidity Analyses

Statistical adjustment for the effects of age and lifetime smoking did not alter the findings of group similarity seen in the unadjusted analyses. Lifetime smoking was categorized as nonsmoking (0 pack-years), moderate (greater than 0 to 10 pack-years) and heavy (greater than 10 pack-years). These results are shown in Table 20-3.

Lifetime smoking consistently exerts significant effects on nearly all historical illness and clinical examination variables, and age was an important factor for the history of pneumonia and the clinical assessment of thorax and lungs (representing an overall clinical assessment of normality/abnormality in the respiratory system), chest asymmetry, the presence of hyperresonance, rales, and the presence of x-ray abnormality.

There were significant or borderline significant group-by-pack-year interactions in analyses of a history of pleurisy and tuberculosis, for the presence of rales on examination, and for x-ray abnormality. There was also an interaction for asthma of borderline significance ($p=0.068$). A significant group-by-age interaction was seen for the presence of rales. The results of analyses stratified to clarify these interactions are shown in Table 20-4.

Nonsmoking Ranch Hands had significantly more asthma ($p=0.050$) than their nonsmoking Comparisons, while the history of asthma was not significantly different in either category of smokers. Pleurisy was significantly more frequent in moderately smoking Ranch Hands ($p=0.0001$), but bordered on being significantly increased in heavily smoking Comparisons ($p=0.060$). Analyses of a history of tuberculosis and the presence of rales was hampered by small numbers of cases in both groups (a total of 13 cases). The presence of several cells containing zeros makes interpretation of these analyses extremely difficult. Except in those strata with zero cells, no statistical significance was noted. In the analysis of x-ray abnormalities, the nonsmoking Ranch Hands had significantly less abnormality ($p=0.030$) than the nonsmoking Comparisons. Analyses of other strata did not reveal any significant group differences.

These adjusted analyses were performed on data from the Original Comparisons, with similar results (see Tables 20-2 and 20-3).

EXPOSURE ANALYSES

The pulmonary data from the Ranch Hands were analyzed using the exposure index as a covariate (categorized as high, medium, or low within each occupational stratum). The percent abnormality at each level of exposure for each clinical or historical variable is presented in Tables 20-5, 20-6, and 20-7.

TABLE 20-1.

Unadjusted Analyses of Reported History of Respiratory Illness by Group

Variable	Statistic	Group: Ranch Hand Number	Ranch Hand Percent	Group: Comparison Number	Comparison Percent	Est. Relative Risk (95% C.I.)	p-Value
Asthma	n	1,016		1,292			
	Abnormal	44	4.3	50	3.9	1.12 (0.74,1.70)	0.58
	Normal	972	95.7	1,242	96.1		
Bronchitis	n	1,015		1,292			
	Abnormal	129	12.7	168	13.0	0.97 (0.76,1.25)	0.84
	Normal	886	87.3	1,124	87.0		
Pleurisy	n	1,016		1,291			
	Abnormal	47	4.6	57	4.4	1.05 (0.71,1.56)	0.81
	Normal	969	95.4	1,234	95.6		
Pneumonia	n	1,016		1,291			
	Abnormal	195	19.2	245	19.0	1.01 (0.82,1.25)	0.89
	Normal	821	80.8	1,046	81.0		
Tuberculosis	n	1,015		1,292			
	Abnormal	7	0.7	6	0.5	1.49 (0.52,4.28)	0.48
	Normal	1,008	99.3	1,286	99.5		