Alterations of Immune Profile among Villagers in Flores, Indonesia*

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SUMMARY Serum total protein, IgM, IgG, IgA, IgE, IgD, C3c and C4, and blood leucocytes were quantitated among residents of five villages in Flores, Indonesia. Levels of total protein, IgM, IgG, IgE and C3c were significantly higher and C4 was lower than in a control group of Indonesians; IgA and IgD did not differ. The control Indonesians had higher levels of IgG, IgA and IgE than a group of Caucasians. People in Flores had higher numbers of circulating eosinophils and lymphocytes, and lower numbers of neutrophils and monocytes than did the Indonesian controls; basophil levels did not differ.

The data collected from persons in Flores were examined for village-to-village variation and the effects of age, sex, malaria parasitaemia, splenomegaly, filariasis *(Brugia timori)* parasitaemia, clinical filariasis (acute or chronic), long-term low-dose chemotherapy with diethylcarbamazine (DEC), ascariasis, hookworm *(Necator americanus)*, gut protozoa, and infection with *Mycobacterium tuberculosis*. The statistical methods employed allowed the analysis of multifactorial variation. Village-to-village variation occurred with eosinophils, lymphocytes and monocytes, but the cause was not apparent. With increasing age, the number of eosinophils, lymphocytes, total protein, IgM, IgE and IgD decreased. Females had higher levels of IgD and C3c. Malaria parasitaemia had no effect. Splenomegaly was accompanied by an increase in IgM, but decreases in neutrophils, eosinophils, lymphocytes and monocytes. *B. timori* microfilaraemia was associated with reduced levels of IgE. In acute filariasis, IgG levels were increased slightly; neutrophil counts increased as disease progressed, but monocyte counts were depressed in all clinically affected people. Persons receiving DEC had less IgM, IgG, IgE, IgD and monocytes, and more C4 than did untreated persons. Ascariasis had no effect. Hookworm infestation was accompanied by decreased numbers of circulating lymphocytes. Gut protozoal infection was associated with lower levels of IgM, but increased numbers of blood neutrophils. Persons with *M. tuberculosis* in the sputum had elevated levels of C4. No effects were seen on IgA or basophils, and no causes of eosinophilia or increased IgE were identified. However, at best only 40 per cent of the variation in parameters could be accounted for. There were significant positive and negative correlations between several variables. No correlation was found between the occurrence of different infections or their clinical expression, in individuals and villages.

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Disturbances of the immune profile have been recorded as sequelae of many tropical infectious diseases and among clinically healthy people living in tropical conditions. Most reports concern the effects of a single organism or disease. The additive or interacting effects of exposure to several disease agents have received inadequate attention.

In Manggarai, the western half of the island of Flores, Indonesia, the residents of certain villages have been observed for several years for expression of diseases. Now, the results of serum protein estimations, including immunoglobulin isotypes, two complement components and blood leucocyte counts, on some of these villagers are reported. The situation allows for comparison of these results with knowledge of the villagers' disease state. In particular, the effects of malaria, Timorian filariasis, tuberculosis, intestinal nematode and protozoal

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infestations, and long-term low-dose treatment with diethylcarbamazine (DEC) are considered.

MATERIALS AND METHODS

Study population

The people, a mixture of Malay and Melanesian stock, comprised residents of six villages (Mahima, Rabo, Karakuak, Waymanis, Wangkung and Sengari) in the Kecamatan (district) of Reo (8°20'S, 120° 30'E) on the northern coast of the Kebupatan (regency) of Manggarai on the island of Flores. The ecology of the area has been described previously.[25,26] Briefly, the population of each village ranged from 80 to 250 people, living in simple wooden houses. The villages were situated either close to the shore or on the slopes above riverine valleys in which rice, corn, cassava and vegetables were grown. The people's diet was poor, deficient especially in animal protein.

Malaria was meso-endemic, caused predominantly by *Plasmodium vivax* and *P. falciparum,* with *P. malariae* and *P. ovale* occurring rarely. Lymphatic filariasis was caused exclusively by *Brugia timori;* the filarial disease pictures in Karakuak and Waymanis have been described previously.[25,26] Prior to chemotherapy, the frequency of microfilaraemia was about 25 per cent and disease was seen in 64 per cent of the villagers. For about three years, the residents of Karakuak, Waymanis, Wangkung and Sengari had been receiving diethylcarbamazine (DEC) at the rate of 50 mg/week for adults and 25 mg/week for children less than 10 years old. The microfilaraemia rate had been reduced to <1%, new clinical cases had not appeared and lesions without irreversible fibrosis had regressed. The residents of Mahima and Rabo had not received DEC. The prevalence of *Mycobacterium tuberculosis* in these villages was 4-37 per cent (H. Danusantoso, D.T. Dennis, D.A. Higgins; unpublished observations). The occurrence of intestinal parasitism among the residents of Mahima, Rabo and Karakuak has been described.[17,27] *Ascaris lumbricoides* occurred in 35-43 per cent, hookworm *(Necator americanus)* in 18 per cent and *Trichuris trichiura* in only 1-4 per cent of the villagers; intestinal protozoal infections were common, occurring in about 50 per cent of the villagers, with *Entamoeba histolytica* and *Entamoeba coli* as the most prevalent species.

Controls

Sera were collected from 20 Caucasian expatriates working in Jakarta and from 22 adult Indonesian residents of Bogor, West Java. Whole blood was collected from 20 Indonesian employees at the Pusat Penelitian Bio Medis, Jakarta. All subjects were clinically healthy and would have suffered minimal, if any, exposure to malaria and filariasis. Clinical samples (blood for parasite examination, stool and sputum samples) from these subjects were not examined.

Clinical examination of Flores residents

A village census, including the personal history of all residents, was taken. Subjects were examined physically, special attention being paid to evidence of filariasis (acute signs: history of episodic fevers, lymphangitis, lymphadenitis, scars over lymph nodes; chronic signs: lymphoedema, hydrocoele, elephantiasis) and spleen enlargement.[15] Blood smears were treated with Giemsa's stain for detection of malaria parasites. To detect microfilariae, 3 ml of citrated blood collected at night was lysed with distilled water and filtered through a Nucleopore membrane, pore size 5 μ. The blood sample was examined microscopically after staining with Giemsa's reagent.[9] Early-morning sputum samples were transported to Jakarta where they were smeared and stained by the Ziehl-Neelsen method and cultured on Löwenstein-Jensen medium to detect *M. tuberculosis.* Faeces samples were obtained from residents of Mahima and Rabo; a modification of the Kato method[20] was used to demonstrate nematode eggs in fresh specimens while material preserved in formalin was transported to Jakarta for identification of protozoa.

Serology

Venous blood was drawn into sterile vacuum tubes without anticoagulant (Vacutainers, Becton Dickinson) and allowed to clot at ambient temperature. In Flores, the clear supernatant sera were carefully pipetted into plastic storage vials about 12 hours after collection, stored for 1-4 days at -4°C, transported on ice to Jakarta, clarified by centrifugation and stored at -20°C. Control sera were decanted, centrifuged and stored at -20°C within 18 hours of collection.

Total serum protein was measured by the Biuret method, modified to compensate for pigment.[31] Immunoglobulin (Ig) M, G, A, E and D and the complement components C3c and C4 were measured by radial immunodiffusion (RID) using commercially available plates and standards (Tri-Partigen, M-Partigen, LC-Partigen, Hoechst Behringwerke). IgE was also measured by radioimmunoassay (RIA) (Phadebas IgE PRIST, Pharmacia); good correlation (± 15%) was seen between RID and RIA; the IgE value recorded was the mean of the two. Total protein, IgM, IgG, IgA, C3c and C4 were calculated as mg/ml; IgE and IgD as IU/ml.

Haematology

Standard methods were used throughout the study.[8] Blood was collected in vacuum tubes containing EDTA (Vacutainers, Becton Dickinson). Total white cell counts were done at the field site within 12 hours of blood collection. Thin smears were made on microscope slides, air dried, fixed in methanol and transported to Jakarta for differential leucocyte counts.

Data analysis

Of the available samples from the Flores survey, the numbers examined for each parameter were: haematology, 576; total protein, IgM, IgG, IgA, IgE, IgD, 300; C4, 180; and C3c, 50. Of the 20 Caucasian and 22 Indonesian controls used for serological comparison, only 15 of each group were examined for C4 and five for C3c. Except for total protein, C3c and C4, the variables were markedly skewed and required transformation to approximate normal distributions; however, the data for IgD, basophils and monocytes included numerous zero values and could not be transformed. Eosinophils, IgM and IgG were logged ($\log_{10}$ (x + 1)). The square root ($\sqrt{x + 0.5}$) of lymphocytes, neutrophils, IgA and IgE was used. After analysis of transformed data, mean values were converted back to the untransformed state for inclusion in Tables.

Most data analyses were done using a computer package for statistics called Genstat.[1] Analysis of variance was used to assess the effect of personal and clinical factors on the variables. The factors were village, age, sex, malaria parasitaemia, spleen size, microfilaraemia, clinical picture of filariasis (none, acute, chronic), DEC treatment, tuberculosis, gut protozoa, ascariasis and hookworms. *Trichuris trichiura* was ignored as only three persons were infected. The filariasis clinical and parasitological classification applied to people receiving DEC was that obtained immediately prior to the commencement of therapy. The programme allowed factors to be fitted sequentially; thus the effect of each factor was assessed after first including the effects of all other contributing factors. Data that could not be transformed were analysed by Kruskal-Wallis one-way analysis of variance,[29] a non-parametric test which analyses ranks rather than actual data, allowing only one factor to be examined at a time. Finally, an examination of possible correlations between the occurrence of diseases was performed using a chi-square test of independence within SPSS.[23]

RESULTS

Comparison of Flores residents with control subjects

Serology. The means and ranges of protein determination for the three groups are given in Table 1. IgG, IgA and IgE were significantly higher in the Indonesian controls than in the Caucasian controls. Residents of Flores had significantly higher levels of total protein, IgM, IgG, IgE and IgD than did both control groups. Their IgA levels were significantly higher than those of the Caucasian controls, but not the Indonesian controls. C3c levels

Table 1 Comparison of serum protein levels between people in Flores and control groups of Indonesians and Caucasians

Group	Protein concentration[a]							
	Total protein	IgM	IgG	IgA	IgE	IgD	C3c[b]	C4[b]
Caucasian controls (n = 20)	74.5 66.5-81.6 (40.5-97.5) $P^1 > 0.1$	1.65 0.9-2.7 (0.60-3.56) > 0.1	14.06 11.6-17.0 (8.24-20.25) < 0.001	1.74 1.3-2.2 (0-3.5) < 0.05	79.6 0-475 (1.55-260) < 0.001	144.3 21.5; 0; 68.7; 7 (0-152) > 0.1	1.090 0.75-1.43 (0.85-1.32) > 0.1	0.220 0.187-0.252 (0.15-0.35) > 0.1
Indonesian controls (n = 22)	78.47 71.4-85.5 (48.0-94.0) $P^2 < 0.01$	1.69 1.0-2.7 (0.54-3.26) < 0.001	19.68 16.4-23.5 (15.01-26.74) < 0.001	2.50 2.0-3.0 (1.09-6.68) > 0.1	1291.3 547-2349 (33-10300) < 0.001	156.3 25.2; 0; 82.6; 7 (0-148.5) > 0.1	1.179 0.92-1.44 (1.0-1.487) < 0.05	0.243 0.216-0.270 (0.176-0.315) < 0.01
Flores residents (n = 300)	89.22 87.4-91.1 (41.0-123.0) $P^3 < 0.001$	4.86 4.4-5.3 (0.54-41.89) < 0.001	31.85 30.4-33.3 (12.03-96.6) < 0.001	2.74 2.6-2.9 (0.92-8.18) < 0.001	3973.2 3588-4378 (39-23800) < 0.001	171.0 39.5; 14.3; 71.0; 57 (0.5-320) > 0.1	0.851 0.79-0.91 (0.384-1.321) < 0.05	0.204 0.194-0.214 (0.051-0.370) > 0.1

P^1 – probability that difference between Caucasian and Indonesian controls was due to chance alone.
P^2 – probability that difference between Indonesian controls and Flores residents was due to chance alone.
P^3 – probability that difference between Caucasian controls and Flores residents was due to chance alone.

[a] Total protein, IgM, IgG, IgA, C3c and C4 in mg/ml, IgE in IU/ml, presented as $\frac{\text{mean}}{\text{95\% confidence limits (range)}}$; IgD presented as $\frac{\text{mean rank}}{\text{median; lower quartile; upper quartile; no. of zeros (range)}}$, thus only the range represents data given as IU/ml.

[b] C3c determinations confined to 15 in each control group and 179 Flores residents; C4 determinations confined to five in each control group and 50 Flores residents.

were significantly lower among Flores residents than among both groups of controls. The C4 levels of Flores residents were significantly lower than those of the Indonesian but not the Caucasian controls. Numerous individuals, irrespective of group, had immeasurably low levels of IgD. Otherwise the only case of immunoglobulin deficiency was a Caucasian male control without measurable IgA.

Haematology. Residents of Flores had significantly higher eosinophil and neutrophil counts and lower lymphocyte and monocyte counts than did the Indonesian controls (Table 2). Numerous zero values were recorded for monocytes (six of 20 controls, 290 of 576 Flores subjects) and basophils (all controls and 559 Flores subjects), thus rendering statistical evaluation unreliable.

Variation within the Flores population

Linear variations are illustrated in Figures 1 (effects of age) and 2 (effect of hookworm) while non-linear variations are summarised in Tables 3 (village-to-village variation), 4 (effect of age, by groups, on IgD), 5 (effects of sex), 6 (effects of splenomegaly), 7 (effects of *B. timori* microfilaraemia and disease), 8 (effects of DEC), 9 (effects of gut protozoa) and 10 (effects of tuberculosis). No variations were attributed to malaria parasitaemia or infestation with *Ascaris* spp.

Total protein. The only significant variation was a decrease, with age, in total protein (Fig. 1).

IgM. Significant effects on IgM levels were noted with regard to age (Fig. 1), spleen size (Table 6), and gut protozoa (Table 9). Thus, IgM levels decreased with age and increased with spleen size; they were reduced in people with protozoal infections. In addition, DEC appeared to have an effect (Table 8); this was difficult to evaluate because the data on gut protozoa were confined to Mahima and Rabo, where the villagers were not receiving DEC. Thus, a significant effect due to protozoa was occurring within the untreated group, but the distribution of this effect among those in the treated group was not known. If a comparison was made of the treated and untreated villages and if the even distribution of protozoal infection was assumed, IgM levels were apparently reduced by DEC.

IgG. Clinical expression of filariasis (Table 7) and DEC (Table 8) affected IgG levels. DEC treatment was associated with reduced IgG levels. For those in the group receiving DEC, the IgG levels were higher in persons exhibiting acute signs of filariasis when compared with the remainder of the population (no signs, chronic signs) as a single group. This significance was not apparent if the three groups (no signs, acute signs, chronic signs) were compared separately, or in the people not receiving DEC; IgG levels were not affected by microfilaraemia, irrespective of the clinical expression of the disease (Table 7).

IgA. None of the factors examined affected IgA levels.

IgE. IgE levels were significantly affected by age (Fig. 1), filarial parasitaemia (Table 7) and DEC (Table 8). All three factors had a negative effect: IgE decreased with increasing age; people with microfilaraemia had lower levels of IgE than did people without microfilaraemia; and people receiving DEC had lower levels of IgE than did untreated people. Since DEC affected IgE levels, the effects of microfilaraemia were examined in the DEC-treated and DEC-untreated groups (Table 7). The trend seen in the whole population (lower IgE levels in people with microfilaraemia) was stronger among people not receiving DEC; it was insignificant, though still present, among those receiving DEC.

IgD. Age (Table 4), sex (Table 5) and DEC treatment (Table 8) affected IgD levels. The relationship with age groups (linear relationship analysis was precluded by the necessary use of non-parametric statistical tests) showed an overall decrease in IgD levels with increasing age. Males had lower levels of IgD than did females. Villagers receiving DEC had lower levels of IgD than did those not receiving treatment.

C3c. Sex was the only factor

Table 2 Comparison of white blood cell counts between people in Flores and a control group of Indonesians

Group	Cell counts[a]				
	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
Indonesian controls (n = 20)	3653.7 3433-3880 (2217-8316)	157.3 151-165 (0-1249)	1.0 0;0;0;20 (0)	2025.3 1898-2160 (815-4655)	281.4 32;0;196;6 (0-220)
Flores residents (n = 576)	2658.2 2548-2771 (316-10905)	598.1 530-652 (0-11811)	13.4 0;0;0;559 (0-172)	3359.9 3236-3486 (315-15922)	214.8 0;0;111;290 (0-740)
	P < 0.001	< 0.001	> 0.1	< 0.001	< 0.1

[a] Neutrophils, eosinophils and lymphocytes as cells/μl of whole blood, presented as $\frac{\text{mean}}{\text{95\% confidence limits (range)}}$; basophils and monocytes presented as $\frac{\text{mean rank}}{\text{median; lower quartile; upper quartile; no. of zeros (range)}}$, thus only the range represents data given as cell counts/μl

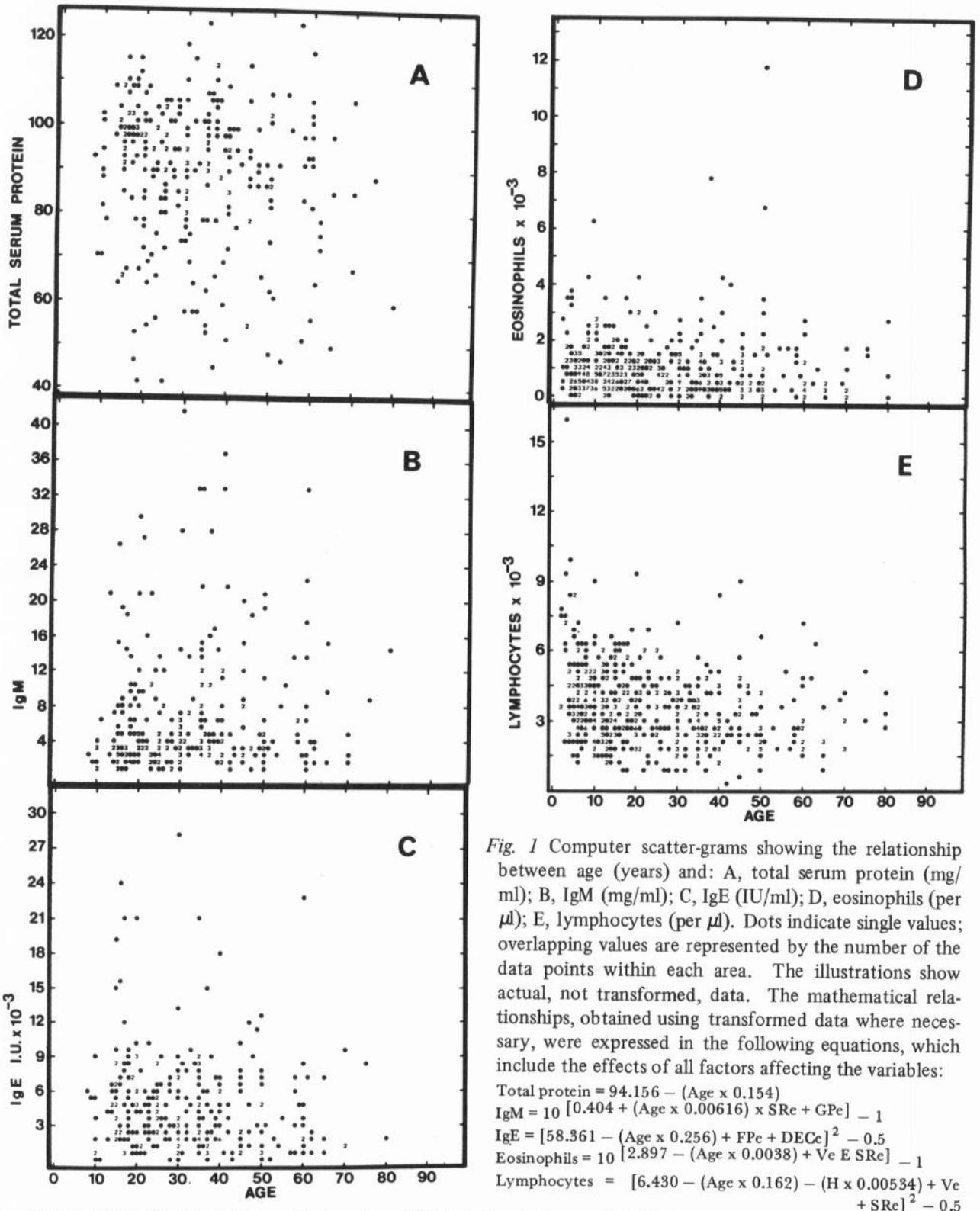

Fig. 1 Computer scatter-grams showing the relationship between age (years) and: A, total serum protein (mg/ml); B, IgM (mg/ml); C, IgE (IU/ml); D, eosinophils (per μl); E, lymphocytes (per μl). Dots indicate single values; overlapping values are represented by the number of the data points within each area. The illustrations show actual, not transformed, data. The mathematical relationships, obtained using transformed data where necessary, were expressed in the following equations, which include the effects of all factors affecting the variables:

$$\text{Total protein} = 94.156 - (\text{Age} \times 0.154)$$

$$\text{IgM} = 10^{[0.404 + (\text{Age} \times 0.00616) \times \text{SRe} + \text{GPe}]} - 1$$

$$\text{IgE} = [58.361 - (\text{Age} \times 0.256) + \text{FPe} + \text{DECe}]^2 - 0.5$$

$$\text{Eosinophils} = 10^{[2.897 - (\text{Age} \times 0.0038) + \text{Ve E SRe}]} - 1$$

$$\text{Lymphocytes} = [6.430 - (\text{Age} \times 0.162) - (\text{H} \times 0.00534) + \text{Ve} + \text{SRe}]^2 - 0.5$$

where SRe = effect of spleen enlargement (spleen sizes of 0-5 for IgM had effects of 0, 0.092, 0.163, 0.355, 0.402, 0.441; for eosinophils 0, −0.0042, −0.104, −0.381, −0.076, −0.534; for lymphocytes 0, 0.8669, −2.493, −6.971, −4.705, −18.320)

GPe = effect of gut protozoal infection (yes = 0; no = 0.156)

FPe = effect of filariasis parasitaemia (yes = 0; no = 10.778)

DECe = effect of DEC chemotherapy (yes = 0; no = 11.286)

Ve = effect of village-to-village variation (for lymphocytes, Mahima = 0, Rabo = −5.190; for eosinophils, Mahima = 0, Rabo = 0.034, Waymanis = 0.155, Karakuak = −0.016, Wangkung = 0.123, Sengari = 0.176).

H = $\log_{10}$ (hookworm egg count − 1)

The effect of age was significant at $p < 0.05$ for total protein and IgE, $p < 0.01$ for eosinophils and lymphocytes, and $p < 0.001$ for IgM.

Table 3 Effect of village on immunological variables

Variable[a]	Village						P
	Mahima (n = 186)	Rabo (n = 85)	Waymanis (n = 54)	Karakuak (n = 128)	Wangkung (n = 29)	Sengari (n = 94)	
Eosinophils	534.5 / 446-640	560.2 / 429-731	770.6 / 552-1077	484.1 / 390-602	676.0 / 428-1066	790.6 / 614-1019	< 0.05
Lymphocytes	3446.7 / 3231-3670	2843.3 / 2556-3145	3038.2 / 2668-3432	3322.4 / 3068-3587	3536.2 / 2996-4121	3881.4 / 3561-4216	< 0.01
Monocytes	321.0 / 59.5;0;155;76	312.0 / 54.0;0;131.7;35	234.1 / 0;0;75.0;35	250.9 / 0;0;72.0;78	319.6 / 63.0;0;152.0;13	269.0 / 0;0;97.0;53	< 0.001

[a] Eosinophils and lymphocytes presented as counts/μl, $\frac{\text{mean}}{95\%\text{ confidence limits}}$; monocytes as $\frac{\text{mean rank}}{\text{median; lower quartile; upper quartile; no. of zeros}}$, not counts.
Variables not listed were not affected

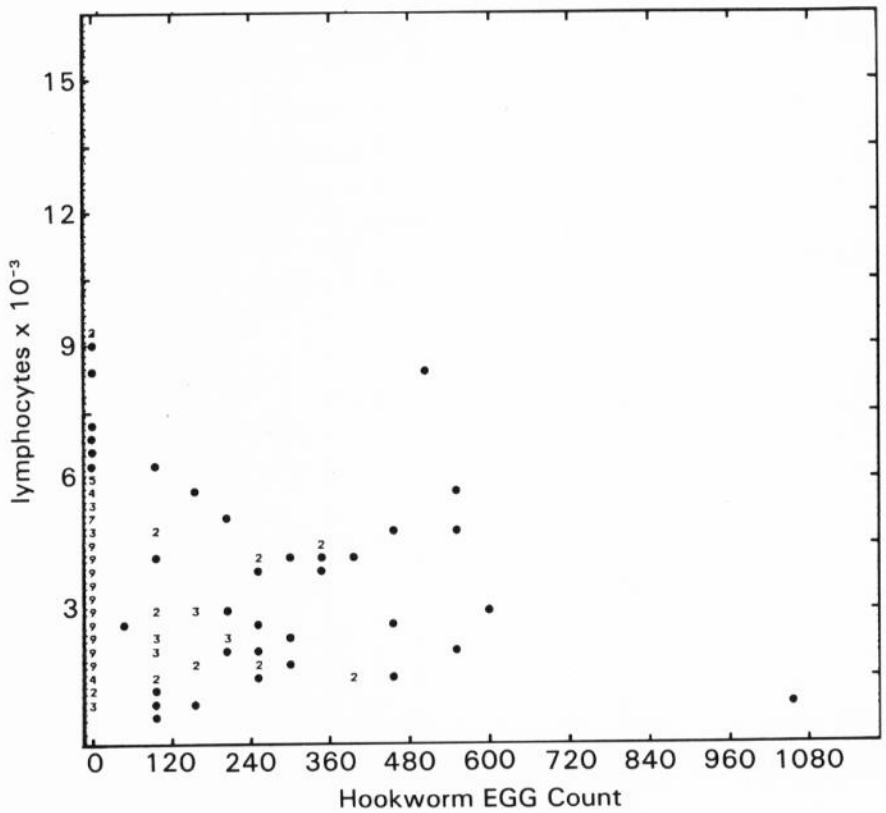

Fig. 2 Computer scatter-gram showing the relationship between hookworm burden (expressed as eggs/g of faeces) and lymphocyte count (per μl). Dots indicate single values; overlapping values are represented by the number of the data points within each area. The illustration is based on actual, not transformed, data. The mathematical relationship between lymphocyte count and relevant factors, derived from transformed data, is given in the legend to Fig. 1. The effect of hookworm infection on lymphocyte counts was significant at $p < 0.05$.

affecting C3c (Table 5), with females having higher levels than males.

C4. DEC treatment (Table 8) and *M. tuberculosis* (Table 10) were associated with significant increases in levels of C4.

Neutrophils. Spleen size (Table 6), clinical expression of filariasis (Table 7) and gut protozoa (Table 9) significantly affected the neutrophil counts. Spleen enlargement was accompanied by a progressive decrease in neutrophil counts. People with acute filariasis had higher neutrophil counts than did people without clinical disease, and people exhibiting chronic signs had still higher counts. Gut protozoal infection was accompanied by increased neutrophil counts.

Basophils. None of the factors examined significantly affected basophil counts.

Eosinophils. There was significant variation in eosinophil counts

Table 4 Effect of age on IgD

Age	n	IgD (mean rank)
0 – 5	0	–
6 – 10	9	170.8
11 – 15	25	195.9
16 – 20	50	159.7
21 – 25	36	148.6
26 – 30	37	146.4
31 – 35	36	143.8
36 – 40	34	113.9
41 – 45	17	158.5
46 – 50	19	129.0
51 +	32	133.6
		$p < 0.05$

with age (Fig. 1), by village (Table 3), and with spleen enlargement (Table 6). Eosinophil counts decreased with age. Residents of Mahima, Rabo and Karakuak had lower eosinophil counts than did residents of the other three villages. As spleen size increased, eosinophils decreased.

Lymphocytes. Lymphocyte counts decreased with age (Fig. 1), with hookworm burden (Fig. 2) and with splenomegaly (Table 6). There was also a village effect (Table 3), examination of which was confined to Mahima and Rabo since data for one of the other significant effects (hookworm) was confined to these two villages: the residents of Rabo had significantly lower lymphocyte counts than did the residents of Mahima.

Monocytes. Village (Table 3), spleen size (Table 6), clinical expression of filariasis (Table 7) and DEC treatment (Table 8) significantly affected monocyte counts. Residents of Mahima, Rabo and Wangkung had higher monocyte counts than did other villagers. A decrease in monocyte count occurred with increased spleen size. Subjects with clinical signs of filariasis had lower monocyte counts than did those without clinical disease, though there was no difference between people with acute and chronic signs. DEC treatment lowered the monocyte count.

Correlations

Between variables. Significant positive correlations occurred between the following: total protein and IgM ($p < 0.01$), IgG ($p < 0.001$) and IgA ($p < 0.05$) levels;

Table 5 Effect of sex on immunological variables

Variable[a]	Males	Females	P
IgD	137.2 34.5;8.75;61.0;33 (n = 152)	159.2 46.5;16.5;77.1;24 (n = 143)	< 0.05
C3c	0.715 0.647-0.784 (n = 25)	0.981 0.915-1.048 (n = 25)	< 0.001

[a] C3c presented as mg/ml, $\frac{\text{mean}}{\text{95\% confidence limits}}$;
IgD as $\frac{\text{mean rank}}{\text{median; lower quartile; upper quartile; no. of zeros}}$, not concentration.
Variables not listed were not affected.

Table 6 Effect of spleen enlargement on immunological variables

Variable[a]	Spleen size						P
	0	1	2	3	4	5	
IgM	3.39 2.9-4.0 (n = 99)	3.67 2.9-4.6 (n = 49)	5.52 4.7-6.5 (n = 85)	7.73 6.3-9.4 (n = 51)	10.11 6.8-14.8 (n = 13)	16.6 6.2-41.9 (n = 2)	< 0.001
Neutrophils	2906.1 2706-3113 (n = 185)[b]	2802.9 2542-3076 (n = 104)	2558.5 2374-2750 (n = 192)	2279.6 1997-2580 (n = 71)	2297.6 1705-2978 (n = 15)	1726.0 546-3567 (n = 2)	< 0.05
Eosinophils	721.4 604-862	698.5 551-885	579.5 487-690	289.1 217-385	538.6 288-1005	151.3 27-837	< 0.001
Lymphocytes	3710.1 3487-3941	3358.6 3077-3653	3299.7 3098-3513	2859.7 2547-3191	2555.7 1936-3261	1199.7 279-2763	< 0.05
Monocytes	289.2 0;0;126.0;97	281.5 0;0;113.5;55	302.5 48.0;0;108.0;86	260.9 0;0;69.0;38	241.0 0;0;53.5;10	241.0 137.0;39.0;196.0;0	< 0.001

[a] IgM given as mg/ml, neutrophils, eosinophils and lymphocytes as no./μl, presented as $\frac{\text{mean}}{\text{95\% confidence limits}}$;
monocytes as $\frac{\text{mean rank}}{\text{median; lower quartile; upper quartile; no. of zeros}}$, not counts.

[b] Group sizes were the same for neutrophil, eosinophil, lymphocyte and monocyte data.
Variables not listed were not affected.

Table 7 Effect of filariasis *(Brugia timori)* on immunological variables

Variable[a]	Group	Microfilaraemia		P	Clinical disease			P
		No	Yes		No	Acute	Chronic	
IgG	DEC-untreated			NS	33.75 30.9-38.9 (n = 88)	39.45 35.0-44.5 (n = 48)	32.59 26.4-40.2 (n = 16)	N vs A vs C > 0.1 A vs N + C < 0.05
	DEC-treated			NS	27.88 25.4-30.7 (n = 53)	28.03 25.6-30.7 (n = 59)	30.73 27.3-34.5 (n = 35)	NS
	All			NS	31.41 29.4-33.6 (n = 141)	32.68 30.3-35.3 (n = 107)	31.31 28.0-35.0 (n = 51)	NS
IgE	DEC-untreated	5508.4 4430-6704 (n = 97)	3346.8 2519-4292 (n = 53)	< 0.001				NS
	DEC-treated	3437.6 2861-4067 (n = 99)	3013.2 2242-3899 (n = 46)	NS				NS
	All	4401.7 3892-4943 (n = 196)	3189.6 2597-3843 (n = 99)	< 0.01				NS
Neutrophils	All			NS	2533.4 2385-2687 (n = 297)	2736.3 2543-2936 (n = 190)	2920.6 2631-3225 (n = 89)	< 0.01
Monocytes	All			NS	308.8 56;0;130;127 (n = 297)	264.8 0;0;91;111 (n = 190)	264.9 0;0;96;52 (n = 89)	< 0.001

NS: Not significantly affected.

[a] IgG given as mg/ml, IgE as IU/ml, neutrophils as no./μl, presented as $\frac{\text{mean}}{95\% \text{ confidence limits}}$;

monocytes as $\frac{\text{mean rank}}{\text{median; lower quartile; upper quartile; no. of zeros}}$, not counts.

Variables not listed were not affected.

IgM and IgG ($p < 0.01$); IgE and IgG ($p < 0.001$); IgE levels and eosinophil ($p < 0.001$) and lymphocyte ($p < 0.01$) counts; IgD levels and monocyte counts ($p < 0.01$); eosinophils and both neutrophils ($p < 0.05$) and lymphocytes ($p < 0.001$); monocytes and both neutrophils ($p < 0.05$) and lymphocytes ($p < 0.001$). Negative correlations occurred between IgM and IgA ($p < 0.05$), IgD ($p < 0.05$) and C3c ($p < 0.001$). Whether these correlations reflected the interdependence of immunological systems or separate parallel responses to common stimuli was not apparent.

Between factors. Possible correlations between the occurrence and expression of filariasis, malaria and tuberculosis were examined. None was observed. There was no correlation between the occurrence of different infections, or their clinical expression, in individuals and villages. It was concluded that the diseases occurred randomly, were not dependent upon known population factors (e.g. sex, village), and that susceptibility (or resistance) to one was not accompanied by susceptibility (or resistance) to others.

DISCUSSION

Comparison of serum protein levels among control groups of Caucasians and Indonesians showed that IgG, IgA and IgE were higher among the Indonesians. A sampling imbalance between Caucasian and Indonesian control groups seems unlikely as the mean ages (Caucasians 33.2 ± 5 years, Indonesians 27.8 ± 5 years) and the sex ratios (Caucasians 10 males, 10 females; Indonesians 12 males, 10 females) of the groups were similar. The levels of IgG and IgE among Cauca-

sians, Indonesian controls and Flores residents might reflect the exposure rate of each community to infectious agents promoting increases in immunoglobulin levels. The rather high levels of IgA in the two Indonesian communities were similar to those recorded among residents of East Java.[11] While racial differences in levels of some immunoglobulins have been described,[19] it is generally believed that IgA levels are not genetically controlled. White blood cell counts of the Indonesian controls were not compared with those of the Caucasians, but the results were similar to most accepted standard values.

Table 8 Effect of long-term, low-dose diethylcarbamazine (DEC) chemotherapy on immunological variables

Variable[a]	Diethylcarbamazine		P
	No	Yes	
IgM	5.44 4.8-6.2 (n = 152)	4.32 3.7-5.0 (n = 147)	<0.01?[b]
IgG	35.32 33.2-37.6 (n = 152)	29.09 26.8-30.5 (n = 147)	<0.01
IgE	4683.4 4094-5313 (n = 150)	3299.9 2800-3841 (n = 145)	<0.001
IgD	163.9 46.5;25.0;75.5;24 (n = 149)	131.7 31.2;6.0;58.5;33 (n = 146)	<0.01
C4	0.191 0.178-0.206 (n = 90)	0.216 0.202-0.229 (n = 89)	<0.05
Monocytes	318.2 58.0;0;146.7;111 (n = 271)	260.1 0;0;86;178 (n = 302)	<0.001

[a] IgM, IgG and C4 given as mg/ml, IgE as IU/ml, presented as $\frac{\text{mean}}{\text{95\% confidence limits}}$;
IgD and monocytes as $\frac{\text{mean rank}}{\text{median; lower quartile; upper quartile; no. of zeros}}$,
not as concentration or cell counts.
Variables not listed were not affected.

[b] Significance of IgM differences could not be assessed due to lack of data on protozoal infection among the treated (yes) group; see text.

Table 9 Effect of gut protozoal infestation on immunological variables

Variable[a]	Gut protozoa		P
	No	Yes	
IgM	7.31 5.9-9.0 (n = 59)	4.63 3.8-5.6 (n = 89)	<0.001
Neutrophils	2482.2 2237-2741 (n = 107)	2852.2 2624-3090 (n = 143)	<0.05

[a] IgM presented as mg/ml, neutrophils as cells/μl, $\frac{\text{mean}}{\text{95\% confidence limits}}$.
Variables not listed were not affected.

The alterations observed among Flores villagers were examined against numerous possible contributing factors. Computer analysis was necessary because of the complexity of the data, and because multiple interactions between factors were expected which could not otherwise readily be examined. An example is appropriate. Preliminary manual analysis suggested that among the villagers not receiving DEC, those with microfilaraemia and acute signs of filariasis had higher levels of IgM (mean 11.2 mg/ml) than any other clinical classification (means 5.3-8.6 mg/ml). This association was refuted by computer analysis. The cause of the discrepancy was an imbalanced distribution of persons with splenomegaly (a factor with a significant effect on IgM) which was compensated for by the computer programmes.

Three "personal" factors were assessed against the variables: age, sex and village of residence. Increasing age saw decreases in total protein, IgM, IgE, IgD, eosinophils and lymphocytes. A decrease in IgD levels with age has been described elsewhere,[28] but the other alterations do not match previous reports.[4,19] Surprisingly, no variable increased with advancing age. The absence of apparent sex-linkage of IgM was surprising; the X chromosome has a strong influence over serum concentration of this protein, resulting in higher levels in females.[19] The occurrence of higher levels of IgD in females has been reported previously.[21] The observation of sex-linkage of C3c levels was contrary to previous studies;[33] such changes might reflect social dif-

Table 10 Effect of tuberculosis on immunological variables

Variable[a]	*M. tuberculosis* in sputum		P
	No	Yes	
C4	0.202 0.188-0.215 (n = 110)	0.290 0.236-0.344 (n = 7)	< 0.01

[a] C4 presented as mg/ml, $\frac{\text{mean}}{95\% \text{ confidence limits}}$.
Other variables were not affected.

ferences between males and females that affected their exposure to pathogens or rates of protein metabolism rather than genetic effects. The underlying causes of village-to-village variation were not clear. They were not, however, due to imbalances in age or sex distributions, or to the concentration of certain diseases or expressions of disease within particular villages. With regard to two of the village-associated variations (eosinophils and monocytes), Rabo and Mahima (where the villagers were not receiving DEC) and one other village (Karakuak for eosinophils; Wangkung for monocytes) were different from the other three villages. If the third village had not been considered, it would have appeared that the effect was associated with DEC therapy. In fact, DEC and village variation were shown to have separate and additive effects on monocyte levels. Other personal factors might be important: nutrition, genetics (racial and familial), tobacco smoking and use of oral contraceptives have been shown to affect cellular and protein immune profile and could be evaluated in the Flores community.

Of the other factors examined, splenomegaly and the filariasis-DEC complex were outstanding in their effects on the immunological parameters. Epidemiological evidence implicating malaria as the cause of the tropical splenomegaly syndrome (TSS) is considerable. The pathogenesis is complex, involving T-cell lymphopenia, imbalance in T-regulator-cell populations allowing polyclonal lymphocyte responses to parasite antigens, massive production of immunoglobulins and formation of immune complexes. IgM is invariably and markedly increased, and increases in IgG and IgA have been recorded.[6,32] In Flores, the only serum alteration accompanying splenomegaly was a rise in IgM. The neutropenia common in TSS[7] was seen in Flores, but accompanied by decreases in eosinophils, lymphocytes and monocytes. Eosinophils probably mature in the spleen[18] so their reduction might be due to an increase in blood volume or a disturbance of splenic functions. No abnormality was associated with malaria parasitaemia, though the number of parasitaemic individuals was small. Differences in immunoglobulin responses during parasitaemia and splenomegaly observed by us and noted in other reports could reflect differences in the host populations or the parasite strains.

Various alterations in serum protein levels have been reported among people with microfilaraemia or disease associated with *Wuchereria bancrofti* and *B. malayi*. In Flores, the only effect of or association with *B. timori* microfilaraemia was a reduction in IgE. IgE can be effective against filarial parasites in antibody-dependent cell-mediated cytotoxicity.[22] Some people remain free from disease and detectable parasites all their lives, yet suffer the same exposure as affected villagers. Thus, it may be asked, can microfilariae survive only in people with lower levels of IgE? Subjects with acute filariasis had elevated levels of IgG, but only when compared to a group amalgamating people with no clinical signs and those with chronic signs, irrespective of their microfilarial status. This contrasts with reports[13,26,30] of increased IgG associated with microfilaraemia or elephantiasis. This might reflect differences in subjects, responses, parasites, experimental approaches, or methods of statistical evaluation. The statistical point is perhaps of singular importance, as emphasised by the observation on IgM levels. As discussed *(vide supra)*, computer analysis disproved an apparent association between combined microfilaraemia and acute filarial disease with elevated levels of IgM. Similar IgM associations reported elsewhere[26,30] might also have represented imbalanced distributions of other factors. Increasing neutrophilia with progression of filaria-induced disease and the reduced monocyte counts among affected people probably reflect pathology and pathogenesis, but their significance is unclear. Surprisingly, no association was found between filarial disease or parasitism and eosinophil counts. Such an association was expected: tropical pulmonary eosinophilia is probably an extreme form of sensitivity to microfilariae, usually accompanied by blood eosinophilia and occult parasitism.

Previous reports of the effects of DEC on immunological variables included decreases in serum C3[3] and either decreases[14] or increases[24] in blood eosinophils a few hours to a few days after high-dose treatment of patients with onchocerciasis or lymphatic filariasis. We attributed decreases in IgG, IgE, IgD and monocytes (and probably IgM), and increases in C4 to DEC. Effects of DEC might occur in two ways. First, by reducing the exposure to parasites; thus, the increase in C4 levels might reflect a reduced utilisation of complement by antifilarial antibodies, or a reduction in

circulating immune complexes, in the absence of antigen. Second, by direct effects on the host; DEC has anti-inflammatory actions which could reduce immunopathogenesis.[16] In view of the inverse relationship between microfilaraemia and IgE, where cause and effect were unknown, it was of interest that after long-term DEC treatment the population levels of IgE had fallen, but people with microfilaraemia prior to treatment still tended towards lower levels of IgE than did their neighbours.

The significant effects of enteric parasites were confined to a decrease in lymphocyte counts accompanying hookworm infection, and increases in neutrophil counts and IgM among people with protozoal infection. A striking aspect of all our data was the absence of factors contributing to the elevations in IgE and eosinophils. All the nematode infections occurring in Flores have elsewhere been incriminated as causes of increases in these two components.[2,5] It is possible that such alterations only accompany intestinal parasitism when the worm burden is heavy, which is rare among the Flores population.

IgG, IgA, IgM and total haemolytic complement might be elevated in active pulmonary tuberculosis.[10] In Flores no immunoglobulin alteration was seen, but people with detectable *M. tuberculosis* infection had elevated concentrations of C4. C4 is an acute-phase reactant and the increases were probably a non-specific indication of active inflammation or tissue injury. Alterations of several acute-phase reactant proteins have been studied among East Javanese with tuberculosis.[12]

This study has confirmed many previous observations and made several new ones concerning the existence and causes of aberrations of the immune profile in tropical areas. Meticulous statistical analysis showed that interactions between factors and their effects were numerous. This emphasises the need for comprehensive analysis of data in the light of all known factors, and indicates that reports of immunological alterations based on knowledge of a single disease or factor are unreliable. Nevertheless, at best we could account for only about 40 per cent of the variation in any parameter. It will be interesting to apply our analytical methods to a more detailed appraisal of the Flores community, incorporating information on genetics, nutrition and serological evidence of viral, bacteriological and parasitic infections. A longitudinal study of infections, disease and chemotherapy in parallel with alterations in serum proteins and blood leucocytes would be informative. It would be particularly interesting to monitor transmigrants moving to areas where filariasis and malaria are endemic.

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