INFECTION AND IMMUNITY, Nov. 1976, p. 1138-1143
Vol. 14, No. 5

Printed in U.S.A.

T-Lymphocyte Number and Function and the Course of Hepatitis B in Hemodialysis Patients

G. C. DE GAST,* B. HOUWEN, G. K. VAN DER HEM, AND T. H. THE
Department of Internal Medicine, University of Groningen, The Netherlands

Received for publication 7 June 1976

To study the relation between general cellular immunity and the course of hepatitis B within a group of chronic hemodialysis patients, T-lymphocyte number and function were investigated in 13 persistently hepatitis B surface antigen (HB_sAg)-positive patients, in 32 HB_sAg-negative patients, and in 11 patients who had recovered from hepatitis B and compared with that of 21 age-matched controls. Phytohemagglutinin-induced lymphocyte stimulation in vitro and the number of circulating T-cells in the HB_sAg-positive group were significantly decreased as compared with those of the recovered group and the controls. Lymphocyte stimulation by pokeweed mitogen and by an antigen cocktail showed the same tendency, but no significant differences between the HB_sAg-positive and the recovered groups. Uremic sera from the three patient groups had similar depressive effects on phytohemagglutinin induced lymphocyte stimulation of controls. Serum immunoglobulin G, (IgG), IgA, and IgM were normal in the three patient groups. It is concluded that chronic hemodialysis patients, who have become persistent HB_sAg carriers, have a significantly decreased T-lymphocyte number and function as compared with hemodialysis patients who are able to eliminate hepatitis B virus (HBV). The difference could not be ascribed to the HBV infection itself. This indicates that T-cells play an important role in the elimination of HBV in hemodialysis patients.

Remarkable differences in the course of hepatitis B between patients and the nursing or medical staff of hemodialysis units have been described (12). Staff members generally have clinical hepatitis with transient hepatitis B surface (HB_s) antigenemia and develop a humoral and cellular immune response against hepatitis B surface antigen (HB_sAg) (5, 22, 25) of which the cellular response is thought to be of prime importance for the elimination of the virus (7). The majority of the patients, however, have either mild or no signs of hepatitis and become persistent carriers of HB_sAg. This has been attributed to the impaired cellular immune status of these patients (7), which has been demonstrated by delayed-type skin reactions (20, 24) and skin graft rejection (4). No studies about the relation between cell-mediated immunity and the course of hepatitis B within the group of hemodialysis patients have been published, however, and factors other than cell-mediated immunity may influence hepatitis B infection in these patients.

In experimental uremia in rats, for example, a decrease in liver cell replication has been observed (3). To eliminate these generally unknown factors and to elucidate the possible role of humoral and cell-mediated immunity in hepatitis B, we studied several immunological parameters in groups of patients with a different outcome of hepatitis B infection, i.e., with clearance or persistence of HB_sAg.

In uremic patients, in vitro lymphocyte stimulation by mitogens and by allogenic lymphocytes have been found to be normal or nearly normal (11, 23). The sensitivity of these tests can probably be increased by using smaller numbers of cells.

We therefore investigated the response of small numbers of cells to low phytohemagglutinin (PHA) and pokeweed mitogen (PWM) concentrations, as well as the response to an antigen cocktail in the presence of control serum. As lymphocyte stimulation in vitro is reduced in the presence of uremic serum (16, 23), we also tested the influence of the patients' sera on PHA-induced stimulation of lymphocytes of controls.

In addition, the absolute number of circulating T-lymphocytes and serum immunoglobulin G (IgG), IgA, and IgM levels were determined.

MATERIALS AND METHODS

Patients. A total of 56 patients on chronic intermittent hemodialysis (dialyzed twice weekly; mean age, 43 years) and 21 healthy control subjects (mean age, 41 years) were studied.

The patients could be divided into three groups in

relation to their response to hepatitis B virus infection. (i) The first group included patients who were HB_sAg positive (n = 13; mean age, 48 years) for longer than 2 years, but HB_sAg negative when they were admitted to the dialysis unit. They had minimal or no liver function disturbances. They never showed anti-HB_s.

(ii) The second group included patients who had always been HB_sAg negative and never had shown anti-HB_s during the period of hemodialysis (more than one year; n = 32; mean age, 41 years).

(iii) The third group included patients who had recovered from hepatitis B infection, as indicated by transient HB_s antigenemia and/or anti-HB_s antibodies (n = 11; mean age, 41 years). Anti-HB_s antibodies were detected transiently (at least on two separate occasions) in seven patients. None showed HB_sAg and anti-HB_s simultaneously at the time of study.

The distribution in sex, creatinine levels of predialysis serum samples, duration of hemodialysis, and underlying renal disease were approximately the same in the HB_sAg-positive and recovered groups. The duration of hemodialysis was shorter in some patients of the HB_sAg-negative group (less than 2 years in 10 patients).

HB_sAg and anti-HB_s antibody determinations. HB_sAg was measured by radioimmunoassay (Ausria 125, Abbott Laboratories). Anti-HB_s antibodies were determined by passive hemagglutination. Sera from the patients were tested twice weekly. The presence of anti-HB_s antibodies in these sera was also determined by radioimmunoassay (Ausab, Abbott Laboratories).

Lymphocyte cultures. Heparinized blood (heparin without preservative) and sera were obtained from the patients just before the regular hemodialysis. Mononuclear cells were isolated by Ficoll-Isopaque centrifugation. Culturing was performed by a modified microculture technique in round-bottom Cooke plates (6). Briefly, 30 × 10 lymphocytes were cultured per well in 0.10 ml of RPMI 1640 with HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, supplemented with 25% pooled human sera from male donors (inactivated for 30 min at 56°C) and 100 U of penicillin per ml and 100 μg of streptomycin per ml. Generally lymphocytes from three patients and one control subject were cultured at the same time.

As stimulants, low concentrations of PHA (Burroughs Wellcome; 1 μl of the stock solution per ml of culture medium) and PWM (Biocult, Grand Island Biological Co.; 1 μg/ml) were used, as well as a cocktail of antigens (purified protein derivative [PPD], candida antigen, diphtheria toxoid, and tetanus toxoid). PHA stimulation of control lymphocytes was also performed in the presence of predialysis sera from the patients. The plates were incubated for 6 days at 37°C with 5% CO_2 in air. Due to the low cell number and the low PHA concentration, the peak response to PHA is delayed until day 6 (our observations). Deoxyribonucleic acid (DNA) synthesis was measured by radioactive thymidine incorporation (labeling with 0.5 μCi of [^{3}H]-thymidine [specific activity, 400 mCi/mmol] during 16 h). Harvesting was performed by an automatic cell culture harvester (Skatron, Oslo, Norway). Incorporated radioactivity was expressed as disintegrations per minute per culture. The arithmetical mean and standard deviation by the antigen cocktail was expressed as the stimulation index (SI) = dpm in antigen cocktail-stimulated cultures/dpm in control culture without antigen. PHA-induced DNA synthesis in the presence of sera from hemodialysis patients was expressed as the percentage of the PHA-induced DNA synthesis in the presence of pooled normal sera.

Absolute lymphocyte and T-lymphocyte numbers. The absolute number of lymphocytes per cubic millimeter was calculated from the leukocyte count (Coulter count) and differential in the counting chamber.

The percentage of T-lymphocytes was determined by counting the percentage of lymphocytes forming spontaneous rosettes with sheep erythrocytes (10). The absolute T-lymphocyte number was then calculated.

Serum immunoglobulin levels. Serum IgA, IgG, and IgM levels were determined by radial immunodiffusion (13).

Statistical analysis. A statistical analysis of the results was performed by the Wilcoxon rank sum test for the lymphocyte stimulation tests and by the Student t test for the number of lymphocytes.

RESULTS

PHA-induced lymphocyte stimulation. As shown in Fig. 1, PHA-induced stimulation was markedly decreased in the HB_sAg-positive group (median, 4,869 dpm) and decreased in the HB_sAg-negative group (8,203 dpm) but not decreased in the recovered group (9,214 dpm) versus the control group (11,431 dpm). PHA-induced stimulation in the recovered group was significantly higher than it was in the HB_sAg-positive group.

PWM-induced lymphocyte stimulation. PWM-induced stimulation (Fig. 2) was decreased in the HB_sAg-positive group (median, 2,013 dpm) but not in the HB_sAg-negative group (4,712 dpm) or in the recovered group (4,579 dpm), as compared with the controls (5,443 dpm). PWM-induced stimulation of the HB_sAg-positive group did not differ significantly from that of the recovered group.

Antigen cocktail-induced lymphocyte stimulation. Thymidine incorporation in control cultures without antigen was nearly the same in the HB_sAg-positive group (265 ± 195 dpm), in the HB_sAg-negative group (315 ± 163 dpm), and in the recovered group (303 ± 145 dpm) as it was in controls (297 ± 111 dpm). Antigen cocktail-induced stimulation, depicted as the SI on a logarithmic scale (Fig. 3), was significantly decreased in the HB_sAg-positive group (median SI, 3.5) and in the HB_sAg-negative group (median SI, 4.6), but not in the recovered group (median SI, 6.6), as compared with that in the controls (median SI, 28.1).

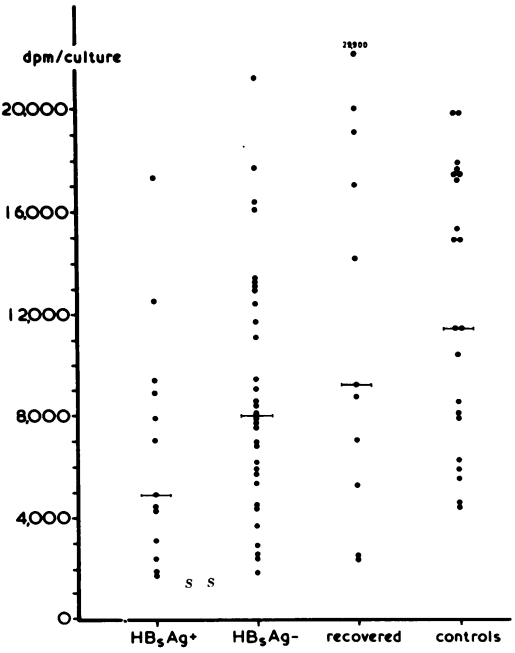

FIG. 1. *PHA-induced lymphocyte stimulation in vitro. Bars indicate median response. PHA stimulation in the HB$_S$Ag-positive group was significantly lower than that in the controls and in the recovered group. The HB$_S$Ag-negative group and the recovered group did not differ significantly from the controls.*

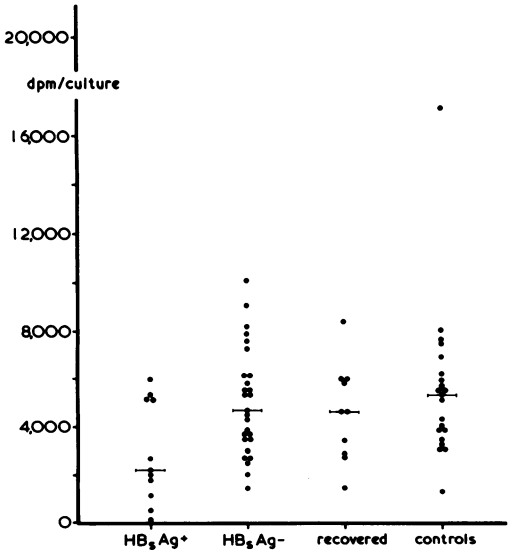

FIG. 2. *PWM-induced lymphocyte stimulation in vitro. Bars indicate median response. PWM stimulation the HB$_S$Ag-positive group differed significantly from that in the controls but not from the recovered group.*

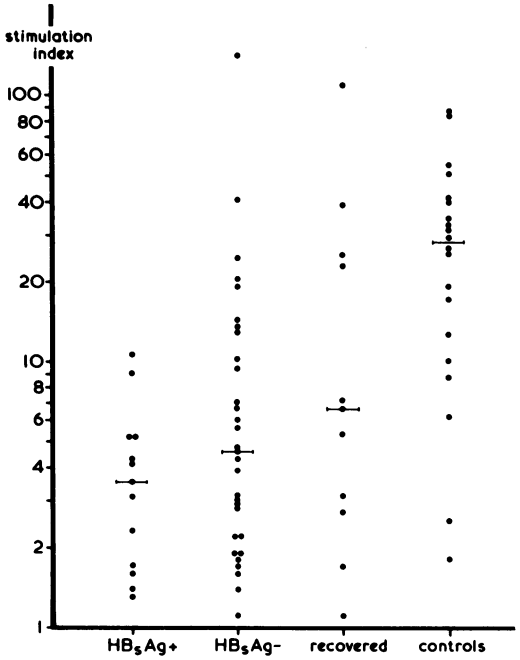

FIG. 3. *Antigen cocktail-induced lymphocyte stimulation in vitro. Bars indicate median response. Antigen cocktail (PPD, candida antigen, diphtheria toxoid, and tetanus toxoid) stimulation expressed as SI was significantly decreased in the HB$_S$Ag-positive and HB$_S$Ag-negative groups, but not in the recovered group, as compared with that in the controls. No significant difference was found between the HB$_S$Ag-positive and the recovered groups.*

The difference in antigen cocktail-induced stimulation between the HB$_s$Ag-positive and the recovered groups was not significant.

Effect of sera from the patients on PHA-induced lymphocyte stimulation. PHA-induced stimulation of control lymphocytes in the presence of sera from the dialysis patients was lower than in the presence of pooled normal sera (average, 57%). The effect of sera from the HB$_s$Ag-positive group (56% ± 23) was not different from that of the HB$_s$Ag-negative group (55% ± 22) or that of the recovered group (62% ± 18).

Absolute lymphocyte and T-lymphocyte number. Figure 4 shows that the absolute number of lymphocytes was markedly decreased in the HB$_s$Ag-positive group (mean number of lymphocytes per cubic millimeter, 1,472) and in the HB$_s$Ag-negative group (1,579), but not significantly decreased in the recovered group (1,945), as compared with that in the controls (2,224). The E-rosette percentages were nearly the same in all groups: 66, 70, 66, and 69%,

respectively. The absolute T-lymphocyte number consequently showed the same picture (Fig. 4, hatched areas): decreased in the HB_sAg-positive group (951) and HB_sAg-negative group (1,102), but only slightly lowered in the recovered group (1,249), as compared with that in the controls (1,552).

The values in the HB_sAg-positive and recovered groups differed significantly.

Serum immunoglobulin levels. Table 1 shows that no differences were found in the serum IgG, IgA, and IgM levels of the patients and those of the controls.

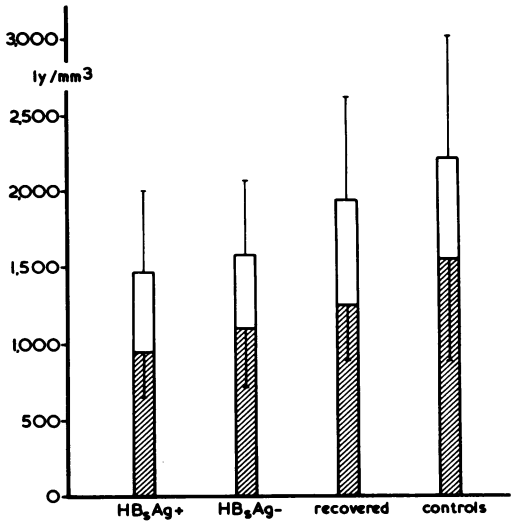

FIG. 4. *Absolute lymphocyte (□) and functional T-lymphocyte (▨) numbers Bars indicate the mean number ±1 standard deviation. Lymphocyte and T-lymphocyte numbers in the HB_sAg-positive and HB_sAg-negative groups were significantly decreased, as compared with the controls ($2P < 0.01$). E-rosette percentages were nearly the same in all groups. T-lymphocyte numbers in the HB_sAg-positive and in the recovered groups differed significantly ($2P < 0.05$). Absolute lymphocyte and T-lymphocyte numbers in the recovered group were not significantly different from those in the controls.*

DISCUSSION

This study showed a relationship between T-lymphocyte number and function and clearance or persistence of HB_sAg within a group of hemodialysis patients. Factors such as duration of hemodialysis, underlying renal diseases, age, or serum creatinine levels were the same in our three patient groups. Until now the difference in the course of hepatitis B between patients and the staff of dialysis units has been ascribed to an impairment of cell-mediated immunity in dialysis patients, but other factors akin to the chronic uremic state were neither distinguished nor excluded.

The great variation in the function and number of circulating lympocytes in chronic hemodialysis patients is reflected in the HB_sAg-negative group. These data, showing a lymphocytopenia and also T-lymphocytopenia and a slight decrease in lymphocyte function, correspond to the literature (11, 23, 24).

When lymphocyte function was regarded in relation to the course of hepatitis B, the group of dialysis patients recovered from hepatitis B appeared to have a normal lymphocyte function, whereas the group of patients with persistent HB_sAg had a significantly decreased lymphocyte function, as compared with the controls as well as with the recovered group. The difference in lymphocyte function between the two patient groups was especially found in PHA-induced lymphocyte stimulation and was not significant in PWM-induced or antigen cocktail-induced lymphocyte stimulation.

In PHA-induced stimulation mainly T-lymphocytes are stimulated, whereas in antigen- and PWM-induced stimulation both T- and B-lymphocytes are stimulated (8). This may indicate that especially T-lymphocyte function was impaired, as a lymphocytopenia is always corrected in lymphocyte cultures and the percent-

TABLE 1. *Serum IgG, IgA, and IgM levels in the different patient groups and controls*[a]

Group	Serum level (mg/100 ml)		
	IgG	IgA	IgM
HB_sAg positives	1,205 ± 321[b]	184 ± 107	140 ± 62
HB_sAg negatives	1,274 ± 329	155 ± 103	144 ± 67
Recovered	1,283 ± 382	175 ± 82	129 ± 68
Control	1,276 ± 321	177 ± 83	128 ± 47

[a] No significant differences between patient groups and controls were found.
[b] Mean ± standard deviation.

age of T-cells was the same in all groups. Another point in favor of a normal B-cell function was the normal serum immunoglobulin levels in all patient groups. The number of circulating T-cells was also within normal limits in the recovered group, but decreased in the HB_sAg-positive group.

An important point is whether the decrease of T-lymphocyte function and number is caused by the hepatitis B virus infection itself. In several viral infections a depression of cell-mediated immunity during the acute phase has been described (18), including acute hepatitis B (1). In healthy carriers of HB_sAg with normal liver function tests, no decrease in cell-mediated immunity was found with dinitrochlorobenzene sensitization (2) or PHA-induced lymphocyte stimulation (21). In contrast to healthy carriers, patients with HB_sAg-positive chronic hepatitis often show an impaired cell-mediated immunity, which may be related to the liver disease rather than to the continuous presence of HB_sAg in the serum.

The group of HB_sAg-positive patients in our study is comparable with the group of healthy carriers because they had no or only a slightly impaired liver function. Consequently, no decrease in cell-mediated immunity can be expected in this group as a result of acute infection or chronic liver disease. Anti-HB_s antibodies were not detected in the HB_sAg-positive group by radioimmunoassay or passive hemagglutination. With these methods anti-HB_s can be detected frequently in the end phase of acute hepatitis simultaneous with HB_sAg and sometimes in chronic HB_sAg-positive hepatitis. This suggests the absence of immune complexes that might depress T-lymphocyte number and function.

In addition, sera from the HB_sAg-positive group had no stronger suppressive effect on PHA-induced lymphocyte stimulation than did sera from the HB_sAg-negative or recovered groups, indicating that HB_sAg itself had no suppressive effect on lymphocyte stimulation in vitro. This is in agreement with previous observations on the effect of plasma of patients with hepatitis B and purified HB_sAg on PHA-induced lymphocyte stimulation (15, 21).

The suppressive effect on lymphocyte stimulation, detected in sera from our patients, is probably due to renal failure products (11, 16, 23) and not to HB_sAg or HB_sAg-anti-HB_s complexes. This is illustrated by our observation in five dialysis patients, tested for cell-mediated immunity before and after infection with hepatitis B, who showed no alteration in their lymphocyte number and function. On the basis of these considerations, it seems unlikely that the impairment of T-lymphocyte function and number was caused by the hepatitis B virus infection. On the contrary, as Dudley et al. have suggested (7), the state of cell-mediated immunity seems to determine the course of hepatitis B infection. In a recent study there was no difference in several immunological parameters between dialysis patients with HB_sAg and patients with anti-HB_s, but a negative association was found in the human histocompatibility antigen HL-A8 with anti-HB_s (19).

Our results suggest that chronic dialysis patients with a nearly normal number and function of T-cells can eliminate the virus, as normal subjects do, whereas patients with a decreased T-lymphocyte number and function can not terminate the virus infection and become persistent HB_sAg carriers.

ACKNOWLEDGMENTS

This work was supported by grant C78 from the Netherlands Kidney Foundation.

We wish to thank Erna Ponds and Mariet Esselink for technical assistance.

LITERATURE CITED

1. **Agarwal, S. S., B. S. Blumberg, B. J. S. Gerstly, W. T. London, J. Millman, A. I. Sutnick, and L. A. Loeb.** 1971. Lymphocyte transformation and hepatitis. I. Impairmant of thymidine incorporation and DNA polymerase activity. Proc. Soc. Exp. Biol. Med. 137:1498-1502.
2. **Bolin, T. D., A. E. Davis, and A. G. Liddelow.** 1973. Liver disease and cell-mediated immunity in hepatitis-associated antigen (HAA) carriers. Gut 14:365-368.
3. **Chen, T. S. N., and C. M. Leevy.** 1973. Liver regeneration and uraemia. Br. J. Exp. Pathol. 54:591-596.
4. **Dammin, G. J., N. P. Couch, and J. E. Murray.** 1957. Prolonged survival of skin homografts in uraemic patients. Ann. N.Y. Acad. Sci 64:967-976.
5. **de Gast, G. C., B. Houwen, and H. O. Nieweg.** 1973. Specific lymphocyte stimulation by purified, heat-inactivated hepatitis B antigen. Br. Med. J. 4:707-709.
6. **du Bois, M. J. G. J., D. R. Huismans, A. Bierhorst-Eylander, M. Groenewoud, P. Th. A. Schellekens, and V. P. Eijsvogel.** 1974. Standardization of lymphocyte cultures in microplates. Tissue Antigens 4:458-463.
7. **Dudley, F. J., R. A. Fox, and S. Sherlock.** 1972. Cellular immunity and hepatitis-associated Australia antigen liver disease. Lancet i:723-726.
8. **Geha, R. S., and E. Merler.** 1974. Response of human thymus-derived (T) and non thymus-derived (B) lymphocytes to mitogenic stimulants in vitro. Eur. J. Immunol. 4:193-199.
9. **Giustino, V., F. J. Dudley, and S. Sherlock.** 1972. Thymus dependent lymphocyte function in patients with hepatitis-associated antigen. Lancet ii:850-852.
10. **Jondal, M., G. Holm, and H. Wigzell.** 1972. Surface markers on human T- and B-lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with SRBC. J. Exp. Med. 136:207-215.
11. **Kasakura, S., and L. Lowenstein.** 1967. The effect of uraemic blood on mixed leukocyte reactions and on cultures of leukocytes with phytohaemagglutinin. Transplantation 5:283-289.

12. **London, W. T., M. Difiglia, A. I. Sutnick, and B. S. Blumberg.** 1969. An epidemic of hepatitis in a chronic hemodialysis unit. N. Engl. J. Med. 281:571–578.
13. **Mancini, G., A. O. Carbonara, and J. F. Heremans.** 1965. Immunochemical quantitation of antigens by single radial immuno-diffusion. Immunochemistry 2:235–256.
14. **Matchett, K. M., A. T. Huang, and W. B. Kremer.** 1973. Impaired lymphocyte transformation in Hodgkin's disease. Evidence for depletion of circulating T-lymphocytes. J. Clin. Invest. 52:1908–1917.
15. **Millman, J., S. S. Agarwal, S. J. Bugbee, B. S. Blumberg, and L. A. Loeb.** 1971. Lymphocyte transformation and hepatitis II. Lack of direct in vitro inhibition by purified Australia antigen. Proc. Soc. Exp. Biol. Med. 138:198–203.
16. **Newberry, W. M., and J. P. Sanford.** 1971. Defective cellular immunity in renal failure: depression of reactivity of lymphocytes to PHA by renal failure serum. J. Clin. Invest. 50:1262–1271.
17. **Nielsen, J. O., V. Reinicke, O. Dietrichson, and V. Anderson.** 1973. Immunological studies of Au antigen carriers with and without liver disease. Clin. Exp. Immunol. 15:9–16.
18. **Notkins, A. L., S. E. Mergenhagen, and R. J. Howard.** 1970. Effect of virus infection on the immune system. Annu. Rev. Microbiol. 24:525–538.
19. **Rashid, A., D. Sengar, W. McLeish, M. Sutherland, J. Harris, and R. Couture.** 1975. The effect of host immunity on hepatitis B antigen (HBAg) infection in hemodialysis patients. Trans. Am. Soc. Artif. Intern. Organs 21:483–487.
20. **Selroos, O., A. Pasternack, and M. Virolainen.** 1973. Skin test sensitivity and antigen-induced lymphocyte transformation in uremia. Clin. Exp. Immunol. 14:365–370.
21. **Sutnick, A. I., S. J. Bugbee, W. T. London, L. A. Loeb, F. Peyretti, S. Litwin, and B. S. Blumberg.** 1973. Lymphocyte function in normal people with persistent Australia antigen. J. Lab. Clin. Med. 82:79–85.
22. **Tong, M. J., A. M. Wallace, R. L. Peters, and T. B. Reynolds.** 1975. Lymphocyte stimulation in hepatitis B infections. N. Engl. J. Med. 293:318–322.
23. **Touraine, J. L., F. Touraine, J. P. Revillard, J. Brochier, and J. T. Traeger.** 1975. T-lymphocytes and serum inhibitors of cell-mediated immunity in renal insufficiency. Nephron 14:195–208.
24. **Wilson, W. E. C., C. H. Kirkpatrick, and D. W. Talmage.** 1965. Suppression of immunologic responsiveness in uremia. Ann. Intern. Med. 62:1–14.
25. **Yeung Laiwah, A. A. C.** 1971. Lymphocyte transformation by Australia Antigen. Lancet ii:470–471.