2. REVIEW OF LITERATURE

2.1. Production of antilymphocyte serum.

Methods of raising ALS have been described by many investigators using lymphoid cells suspended in a medium or emulsified in complete Freund's adjuvant. Many immunization techniques have been used yielding ALS of high immunosuppressive potency. The most common are the two or three subcutaneous or intravenous injections given at two weeks apart followed by a booster immunization four weeks thereafter. Many different host species have been used to raise effective ALS. The most used species are the rabbit and the horse. Pigs and ruminants have also been used but they produce antisera of lower quality.

Antisera have been raised against lymphoid tissues of human, monkey, dog, rat, bovine and mouse.

The production of anti-rat ALS in rabbits was reported by Woodruff and Anderson (1963). They injected intraperitoneally $200.0 \times 10^6$ rat thoracic duct lymphocytes three times at weekly intervals. The rabbits were bled ten days after the last injection and booster intraperitoneal injection of $1-2 \times 10^6$ lymphocytes were given ten days before each subsequent bleeding.

The production of anti-mouse ALS was communicated by Levey and Medawar (1966a), Gray et al. (1966) and Monaco et al. (1966a). Levey and Medawar (1966a) administered
two intravenous injections of $4 \times 10^8$ lymphoid cells in rabbits at fourteen days apart. The rabbits were then bled 7 days after the second injection and the sera were collected and heat inactivated (56°C for 30 minutes), Seitz filtered and stored at -20°C until used. Gray et al. (1966) and Monaco et al. (1966) reported their method of raising ALS in rabbits as follows. Each rabbit received a total of $100.0 \times 10^6$ lymph node cells emulsified in complete Freund's adjuvant. The emulsion was equally divided and injected into each of the four footpads. The booster injections of saline cell suspension of $100.0 \times 10^6$ cells without adjuvant were given intravenously on three successive days four weeks later. The rabbits were bled four days after the last injection. The blood was left to clot at room temperature and serum was collected the next day, heat inactivated and stored at -20°C.

The mode of production of ALS in horses was reported by a number of workers. Iwasaki et al. (1967) immunized ten horses with dog's splenic and lymph node cells and 2 horses with human lymphoid tissues. The horses were given 4 to 6 inoculations at weekly intervals with 0.2 to 194 billion lymphoid cells in saline. They found that the sera when injected into dogs caused lymphopenia. They observed that doses of up to 194 billion lymphoid cells would rapidly increase the cytotoxic titer. Balner et al. (1968) and Sheil et al. (1971) injected horses by the subcutaneous route with $1.0 \times 10^{10}$
lymphoid cells on 4 to 6 occasions at 2 to 4 weeks intervals for the production of anti-human ALS. The horses were bled one week after the last injection. They reported that sera produced in this manner prolonged skin graft survival in monkeys. Lucke et al. (1968) immunized horses by 3 subcutaneous injections of $1.0 \times 10^9$ pig lymph node cells in medium 199 at 7 days. Blood was collected one week after the last injection. Twenty four hours thereafter the horses were given another injection of $0.44 \times 10^9$ lymph node cells and bled 7 days later. The blood was allowed to stand for 2 hours at room temperature and then kept overnight at $4^\circ$C. The serum was separated, absorbed with one third of its volume with pig erythrocytes and sterilized by Seitz filtration and stored at $-20^\circ$C until used. They observed that sera produced in this manner were effective and prolonged skin graft survival in pigs. Carroll et al. (1976) immunized 2 horses by two subcutaneous injections of $2.0 \times 10^5$ bovine milk leucocytes incomplete Freund's adjuvant followed by intravenous injections of leucocytes suspension in saline every week thereafter until 51 week. Blood was collected every week. The horse antobodyne leucocyte serum when injected into cattle caused neutropenia within 1 hour.

Binns et al. (1971) reported the technique of production of anti-mouse, anti-rat, anti-dog and anti-chicken ALS raised in either pigs, calves, one goat or sheep. Each of the animals received varying quantities of donor thymic cells in saline suspension or emulsified in complete Freund's adjuvant. Most
animals were given two injections at two week intervals. They observed that the method developed for the production of immunosuppressive serum in the pig against mouse lymphocytes proved satisfactory for the preparation of anti-rat, anti-dog and anti-chicken ALS. Bovine, sheep and goat ALS made against mouse thymocytes also had strong immunosuppressive activity.

In the bovine, production of immunosuppressive activity seemed age dependent. They noted that poor immunosuppressive activity was found in calves which were less than 2 months old.

The production of anti-mouse, anti-human, anti-dog and anti-guinea pig ALS in goats has been reported by Rolland et al. (1971). These animals received four intravenous injections of $5.0 \times 10^7$ cells per kg body weight at weekly intervals and were killed and bled out one week after the last injection. They found a correlation between the in vitro membrane immunofluorescence titer of the sera against mouse thymocytes to the in vivo immunosuppressive activity as judged by prolongation of tail skin homograft survival.

Sheil et al. (1971) communicated the production of anti-human lymphocyte serum in 3 goats. Each goat received $5.0 \times 10^9$ thymocytes in multiple subcutaneous injections on 3 occasions. The first immunization was followed after 2 weeks by 2 injections at weekly intervals. One week after the third injection the goats were bled out and 1.5 liter of serum was obtained from each. The antilymphocyte globulin prepared from this ALS
was immunosuppressive in monkeys with skin xenograft.

Effective anti-mouse ALS in chickens and ducks was produced by Joo et al. (1968). They found that in vitro the sera were strongly cytotoxic to mouse lymphocytes in the presence of duck or rabbit complement but injection into mice brought about only minor transient lymphopenia.

Mechanism of action of ALS.

Many hypotheses have been proposed on the mode of action of ALS. Most of these hypotheses have been tried out and discarded and only the hypothesis proposed by Lance (1968) was acceptable. Lance found that the globulin fraction of ALS was active in immunosuppression. He reported that the anti-lymphocyte globulin achieved its immuno-suppressive action by causing selective depletion of cells of the recirculating pool of lymphocytes. This was supported by the findings of several investigators like Dennan and Frenkel (1968) who studied the distribution of ALS in rats and mice by immunofluorescence and \(^{131}\)I labeling. The results suggested that the ALS penetrated the thymus, spleen and lymph nodes to a limited extent. These findings explained the selective immunosuppressive action of ALS. In another experiment Dennan and Frenkel (1968) found that the thymus weight decreased in conjunction with a persistent lymphopenia. They concluded that this could be explained by the release of small lympho-
cytes into the circulation. Lymphopoiesis in the thymus and spleen was not inhibited by ALG and plasmacytosis was noticed in the spleen. These findings supported the idea that ALS mainly acted on the peripheral blood lymphocytes and the immune function of these cells was suppressed.

The mechanism of action of the active component of ALS was studied in mice by Lancé (1969a) who used an antibody eluate prepared by absorption and subsequent elution from thymocyte membrane. The resulting antibody eluate was labelled with $^{125}$I and injected into mice which were killed at intervals and their tissues examined by radiography to determine the antibody activity. The result indicated that the labelled antibodies were eliminated from the recipients extremely rapidly. The mechanism of this rapid clearance appeared to depend upon the absorption of antibody molecule on the lymphocyte surface and the subsequent clearing and degradation of the antibody-lymphocyte complexes by the reticuloendothelial system. Distribution studies confirmed that the major site of the antibody lymphocyte interaction was in the peripheral blood with relatively little penetration of antibody within lymphoid organs. Radiographic studies showed that the pattern of localization within lymphoid and other organs was confined to rather specific areas. These observations were believed to offer strong support for the notion that ALS achieved its immunosuppressive effect through a selective reduction of the
population of recirculating lymphocytes.

From their studies on the mechanism of action of ALS, Everett et al. (1970) demonstrated that ALS selectively destroyed long lived small lymphocytes, a population containing the immunocompetent cells. This finding supports the suggested mechanism of action of ALS by Lance (1968).

The effects of ALS in vitro.

The effects of ALS in vitro have been described by several investigators. Gray et al. (1966) reported that incubation of lymphocytes with ALS caused agglutination of these cells. They observed that incubation of lymphocytes in undecomplemented rabbit anti-mouse lymphocyte serum (RAMLS) killed approximately 50% of mouse spleen or lymph node cells. Heating to 56°C for 30 minutes removed the cytotoxic effect and addition of fresh guinea pig complement to the decomplemented RAMLS restored the cytotoxic activity. Abaza and Woodruff (1966) and Woodruff (1967) communicated that ALS and its bivalent pepsin fragments F(ab)2 agglutinated lymphocytes, while Monaco et al. (1967) and Brent et al. (1967) reported that purified 7S gammaglobulin fraction of rabbit anti-human lymphocyte serum (RAHLS) and RAMLS agglutinated and killed lymph node cells and peripheral blood lymphocytes in vitro. Equally, Gray et al. (1966), Abaza and Woodruff (1966) and Iwasaki et al. (1967) found that ALS which had been inactivated by heating to 56°C for 30 minutes agglutinated lympho-
cytes and destroyed lymphocytes in the presence of complements.

Greaves et al. (1969) found a correlation between the suppression of skin graft rejection and agglutinating, cytotoxic, mitogenic and opsonizing properties of ALS. They observed that the opsonization titer gave the best correlation with immunosuppression.

Bach and Antoine (1968) and Bach (1970) reported that lymphocytes, after incubation with ALS at 37°C for 90 minutes, lost their capacity to form spontaneous rosette with SRBC. They observed that the inhibition could take place in complement-free medium, but the sensitivity was much increased by complement. Bach (1970) found a positive correlation between rosette inhibition titer of ALS and immunosuppressive activity, but no good correlation was observed between cytotoxicity titer and rosette inhibition titer. Rosette inhibition was obtained at nontoxic concentration of ALS. Paraskevas et al. (1972) studied the reaction of ALS with lymphocytes in vitro using the technique of reverse immuno-cytotoxic adherence which detected surface gammaglobulin through the formation of rosettes. They observed that ALS blocked the detection of gammaglobulin in 30 - 40% of mouse spleen lymphocytes. This was shown by inhibition of rosette formation. The 7S or 19S fractions used separately or combined, also blocked the surface gammaglobulin.

Rolland et al. (1971) reported that incubation of mouse thymocytes with conjugated anti-mouse antilymphocyte globulin showed membrane fluorescent staining of the thymocytes. They
observed that there was a correlation between the membrane immunofluorescence titer and mean graft survival time.

2.4. The effects of ALS in vivo

2.4.1. Effects on the circulating white blood cells.

Generally after ALS injection there is a slight depression in the leucocyte counts, but the absolute number of lymphocytes is significantly reduced. Gray et al. (1966), Brent et al. (1967) and Tursi et al. (1969) working with mice reported that a single intraperitoneal injection of RAMLS caused a profound drop in the absolute number of lymphocytes in mice. The total white blood cell counts were slightly depressed, but this was entirely due to the disappearance of lymphocytes. However, the absolute numbers of polymorphonuclear leucocytes and other cells were increased. They observed that ten days after the injection or RAMLS the total white cell counts were normal, but the differential counts showed this to be still due to an increase in polymorphonuclear leucocytes and other non-lymphocyte leucocytes. The total lymphocyte counts only reached 60% of the preinjection level. Two to three weeks after the injection of RAMLS Tursi et al. (1969) noted that the lymphocyte count gradually returned to normal. The same change in the blood picture was also observed in rats by Agnew (1968) and Curry and Ziff (1966).

Iwasaki et al. (1967) studied the effects on the peri-
pheral blood lymphocytes of dogs and humans treated with ALS. They noted that lymphopenia was produced after ALS administration. The diminution of the absolute number of lymphocytes however, was less striking because of the increase in the total white cell counts. There was often an increased number of immature granulocytes. When dogs were injected with unabsorbed ALS to red cells, acute anemia developed.

Monaco et al. (1967) reported that human patients injected with rabbit anti-human lymphocyte serum (RAHLS) developed profound, but transient peripheral lymphopenia. The absolute number of lymphocytes reached 10 - 20% of the pre-injection level and persisted for 2 - 4 days after the last injection. They observed an invariable transient granulocytosis, but no fall in haematocrit and platelet counts. No change in lymphocyte counts was observed in patients with chronic lymphatic leukemia when treated with RAHLS and they presumed that this was due to the relatively small doses of RAHLS as compared with the large number of peripheral and tissue lymphocytes.

Hay et al. (1974) working with sheep reported that rabbit anti-sheep lymphocyte serum (RASLS) and rabbit anti-sheep lymphocyte globulin (RASLG) given locally either by subcutaneous injection or by endolymphatic infusion, both preparations almost completely eliminated recirculating lymphocytes from the efferent lymph of the regional node. When given intravenously, the ALS caused a profound lymphopenia and the
level of lymphocytes in the blood was reduced to about ten per cent of the pretreatment value within a period of 4 to 5 days. This lymphopenia persisted for several weeks without any further injection of ALS. As the level of lymphocytes in the blood fell, there was a concomitant fall in the concentration of lymphocytes in the lymph and in the output of lymphocytes from the peripheral lymph nodes. They also observed that administration of RASLS or RASLG, either locally or intravenously, had no apparent effect on the production of blast cells or antibody-forming cells within the popliteal lymph node in response to challenge with Salmonella antigens. These cells appeared in the lymph in normal numbers. They observed that even when more than 99 per cent of the circulating lymphocytes was eliminated from the lymph, cells within the lymph node could react to antigen and give rise to essentially normal numbers of blast cells and antibody forming cells. The administration of ALS had no effect on the traffic of polymorphonuclear cells out of the node. Because of the restricted action of ALS on recirculating lymphocytes, it can be used in vivo to obtain large numbers of antigen-stimulated cells, antibody-forming cells, or polymorphonuclear leukocytes uncontaminated with small lymphocytes.

Elliot et al. (1978) reported that rhesus monkeys infused with antihuman thymus globulin (AHTG) raised in horse showed a rather marked transient anemia as shown by the de-
crease in erythrocytes, packed cell volumes and haemoglobin and by the increase in reticulocytes. This effect, however, was abrogated by absorbing the serum with human erythrocytes. They noted that 24 - 48 hours following AHTG infusion the total leukocyte counts were decreased. However, the absolute neutrophil count was elevated.

Effect of ALS on the tissue histology.

Taub and Lance (1968b), Denman and Frenkel (1968) and Taub (1969) reported the histological changes in mice and rats after ALS injection. Taub and Lance (1968a) and later Taub (1969) showed that after administration of a single subcutaneous dose of RAMLS, a well defined sequence of changes occurred in mice lymphoid tissues. Between 12 and 24 hours after injection damaged lymphocytes with pyknotic nuclei appeared within the capillaries and venules in the para-cortical areas of the lymph nodes and in intestinal Peyer's patches. By 48 hours a striking depletion of small lymphocytes sharply localized to para-cortical areas of lymph nodes was seen. These regions were adjacent to or distant from the site of injection. Within the para-cortical areas a few small lymphocytes remained closely adjacent to post capillary venules, but the majority of cells remaining in this area were large lymphocytes, a few immuno-blasts and reticular cells. They observed that after three repeated
doses, areas of depletion in small lymphocytes developed in the spleen. These were sharply restricted to the periarterial sheets of lymphoid follicles. A collection of immunoblasts usually persisted as a rim of cells just surrounding the central follicular arterioles. Except for the decrease in weight, they found that antiserum administration did not affect the thymic architecture and cellular population, even when the lymph nodes and spleen had been severely depleted of lymphocytes. They also noted that multiple injections usually induced a hyperplasia of erythroid elements in the bone marrow.

After 3 months of weekly treatment of ALS or normal rabbit serum most animals developed histologic evidence of nephritis with fibrinoid accumulations within the glomeruli and thickening of Bouwman's capsule similar to lesions seen in serum sickness nephritis. Denman and Frenkel (1968) found that after ALS administration lymph nodes were uniformly enlarged and loss of architecture, lymphocyte depletion and plasmacytosis were evident. The percentages of blast cells and plasma cells were comrisingly high in the lymphopenic rats. In the spleen there was severe depletion of lymphocytes and in the thymus there was reduction of cortical thickness accompanied by decrease in weight.

Iwasaki et al. (1967) found lymphoid hyperplasia in dogs treated with anti-dog lymphocyte plasma, serum globulin, particularly in animals treated for 2 months or longer. They observed that lymph nodes were normal or enlarged. The cortex
contained numerous pyroninophilic cells similar in structure to those encountered in the splenic follicles. The number of small lymphocytes surrounding the lymph follicles were greatly reduced. The medulla contained varying numbers of smaller, deeply pyroninophilic cells and some plasma cells. They reported that in the spleen there was follicular hyperplasia and the follicles were bigger and more numerous than in untreated dogs. The follicular centers were crowded with large and medium sized cells with lightly pyroninophilic cytoplasm and large pale nuclei with prominent nucleoli and many of these cells were in mitosis. Some reticular cells and macrophages contained ingested nuclear debris. In the kidney of dogs treated with anti-dog lymphocyte sera they observed periodic acid Shiff (PAS) positive thickening of the glomerular capillary basement membrane.

Monaco et al. (1966) reported that mice continuously treated with RAMLS became severely wasted, showed progressive weight lose, hunching of the back, severe alopecia and a variable degree of diarrhea. All mice died 42 to 56 days postgrafting. Postmortem examination of these animals showed profound atrophy of the lymphoid organs particularly the lymph nodes. Areas of coagulation necrosis in the liver and spleen were common.

Gray et al. (1966) reported that consistent changes were found in the lymphatic tissues in mice injected with
RAMLS. The thymus was depleted of lymphoid cells but epithelial elements were preserved. The Peyer's patches showed decreased number of lymphocytes which were found to be less tightly packed and replaced by histiocytes and macrophages. The most pronounced changes were apparent in the lymph nodes and to a lesser extent in the spleen. One week after RAMLS administration there was a marked diminution in the number and size of germinal centers in the nodes and to a lesser extent in the spleen. After one week of treatment many nodes were severely depleted of lymphoid cells and evidence of cell death was present in the form of cell debris and pyknotic nuclei. They observed that two weeks after termination of serum treatment the lymph node and spleen showed evidence of recovery with repopulation of lymphoid cells and reappearance of germinal centers.

Elliot et al. (1978) reported that in monkeys infused with anti-human lymphocyte serum the lymph nodes became smaller and the cortical area was less densely populated. The lymph nodes and spleen were often depleted of small lymphocytes in the so-called thymus dependent areas. They observed a rather consistent finding of thrombophlebitis in the femoral and saphenous veins.

The effect of ALS on graft survival.

Generally the efficacy of ALS was assayed by its ability to prolong graft survival time. Monaco et al. (1966, 1966a)
reported that mice given daily RAMLS for one week prior to grafting showed a significant prolongation of graft survival between 21 and 24 days. They observed that the rejection corresponded to the time when repopulation of the lymph nodes had occurred. When the mice were injected daily with RAMLS for 7 days prior to grafting and then continuously five time a week after grafting the skin graft showed no sign of rejection at any time. After 5 weeks of continuous treatment, however, the animals became severely wasted and eventually all died 42 to 56 days postgrafting with all skin graft remaining in perfect condition. They also found that RAMLS significantly inhibited second-set allograft rejection response. Levey and Medawar (1966, 1966a) communicated that the administration of RAMLS was most effective on the second and fifth day after grafting. In this manner they obtained an average graft survival prolongation of 40 days. They also found that RAMLS abrogated second set graft response and concluded that this property distinguished ALS from all immunosuppressive agents. Under continuous ALS treatment Levey and Medawar (1968) and Lance and Medawar (1969) observed that mice could accept skin heterograft from donors of very distant genetic relationship e.g. guinea pig, rabbit or human.

Woodruff and Anderson (1963) reported that rats receiving daily treatment of rabbit anti-rat lymphocyte serum (RARLS) starting on day 7 prior to grafting until 2 weeks thereafter had an average skin graft prolongation of 28 days. They found
that combination of lymph drainage from day -5 to day 0 and daily RARLS treatment after grafting for 14 days resulted in an average graft survival time of 35 days. Anderson et al. (1967) observed that the F(ab')2 and Fab' fragments of horse anti-rat IgG had no effect on the homograft survival time. Besides they found that rabbit anti-rat IgG gave a mean survival time of 28 – 35 days and horse anti-rat IgG only 20.8 days. The graft survival time of control rats and rats receiving normal horse and rabbit serum was 8 days.

Malek et al. (1969) performed skin allografting in 15 dogs followed by daily treatment with horse anti-dog lymphocyte serum (HADLS) for 4 weeks or until graft rejection. The dogs were divided into three groups, consisting of dogs receiving HADLS intravenously, dogs receiving HADLS subcutaneously in the right foreleg and subcutaneously in the left hindleg. They found that in the group of dogs receiving the HADLS on the grafted leg, the graft showed no evidence of rejection throughout the 4 week observation period. In dogs receiving HADLS intravenously and subcutaneously far from the graft, however, the skin grafts were rejected within 8 – 18 days.

Skin graft survival of 18 – 20 days was observed in monkeys treated subcutaneously with anti-human lymphocyte serum by Balner et al. (1968), Lance and Medawar (1970) and Balner (1972). Lance and Medawar (1969) found a longest prolongation of 20 days in the heterografts as compared to 45
days prolongation in homografts.

Sheil et al. (1971), Najarian and Simmons (1971), Najarian et al. (1976) and Thomas et al. (1977) reported that antihuman lymphocyte globulin injected into patients with renal transplant gave a successful graft prolongation. There was a much higher survival of renal transplant in patients receiving ALG as compared to patients without.

Effect of ALS on the humoral antibody response.

It has been shown that ALS inhibits the humoral antibody response in mice and rats. Monaco et al. (1965b, 1966), Denman et al. (1966) and Barth et al. (1968) reported that RAMLS depressed the primary immune response to SRBC in mice. They observed that with the increase in number of injections of RAMLS a more relevant depression of the humoral antibody response was achieved. Lance (1970) found that the depression of the primary immune response was especially effective if ALS was given prior to the antigen. He observed that the magnitude of the depression varied directly with RAMLS dose and inversely with the antigen dose.

Curry and Ziff (1966) observed a complete suppression of antibody response to SRBC in rats receiving rabbit antirat lymphocyte globulin. But the rats developed antibody to rabbit globulin. James and Milne (1971) demonstrated complete suppression of the primary immune response to SRBC, bovine serum globulin, and human lymphocytes.
albumin (BS') and pneumococcus type II polysaccharide inoculation in mice injected with RAMLG. They found that the suppression varied from one mouse strain to the other.

Monaco et al. (1966) and Lance (1970) observed that ALS administration had lesser effect on the secondary immune response. On the other hand Monaco et al. (1967) found that human patients injected with RAHLS formed demonstrable antibody to rabbit gammaglobulin. One patient showed serum sickness 2 - 3 weeks after antisera injection with high antirabbit globulin titer at that time.

Effect of ALS on the responsiveness of lymphocytes to phytohaemagglutinin (PHA).

The responsiveness of lymphoid cells from ALS treated mice was reported by Tursi et al. (1969). They observed that thymus, lymph node and peripheral blood lymphocytes taken from mice treated with ALG showed a greatly diminished response to PHA in vitro. Recovery of the circulating lymphocyte level preceded recovery of responsiveness to PHA. The responsiveness to PHA could be prevented by re-injection of ALG or thymectomy. They observed that after PHA responsiveness had recovered to the value of approximately 20% of the normal, graft rejection also began to take place. They concluded that the effect of ALS on the thymus dependent lymphocytes in mice could be monitored
by assessing the PHA sensitivity of the peripheral white blood cells.

The effect of ALS on the delayed hypersensitivity reaction.

Interbitzen (1956) and Waksman et al. (1961) reported that BCG sensitized guinea pigs receiving ALS showed a marked depression or abolition of the tuberculin reaction. Waksman et al. showed that the suppressive effects of a single dose of ALS on tuberculin reaction was clearly related to the time at which the ALS was administrated. They noted that the affected tuberculin reaction was caused by a diminution in the amount of cellular infiltration in the skin. They also observed that the delayed reaction to purified diphtheria toxoid was reduced or completely suppressed in guinea pigs treated with ALS. The suppressive effect was observed in guinea pigs that had lymphocyte counts below 4000/mm³. Human patients infected with RAHLS showed no delayed hypersensitivity skin reaction to tuberculin as reported by Monaco et al. (1967).

The effect of ALS on the B and T lymphocytes.

The effect of ALS on the B and T cells was reported by Bishop et al. (1975), Cosimi et al. (1976), Thomas et al. (1977) and Thomas et al. (1978) in human and monkeys. They observed that anti-human thymocyte globulin (AHTG) injection
caused a profound fall in the T lymphocytes to below 10% of the pretreatment level. Thomas et al. (1977) found that this effect persisted for more than one week after AHTG administration. AHTG administration was associated with rises in surface immunoglobulin-positive (B) cells to 72 to 93% and EAC receptor positive cells to 36 to 68% of the mononuclear cells, indicating the selective effect of AHTG on the circulating T cells. Thomas et al. (1978) suggested that the immunosuppressive potency of rabbit AHTG in the primate skin graft assay was related largely to its anti-T cell activity. Bishop et al. (1975) proposed that the monitoring of circulating rosetting (T) cells may be a useful clinical guide to the degree of T cell immunosuppression.