DISCUSSION

Interleukin 2 (IL-2; T cell growth factor) is produced by T lymphocytes, stimulates cells from haemopoeitic lineages which express specific IL-2 receptors to proliferate (Robb, 1984). The proliferation is generally measured by ^{3}H-thymidine incorporation (Smith and Ruscetti, 1981). The IL-2 dependent cell lines and short-term lectin-induced lymphoblast are widely used as target cells in measuring IL-2 activity. Cell lines commonly used include CTLL and HT-2 (Kappler and Marrack, 1986). Short-term lectin-induced lymphoblast are from Con A or PHA stimulated T cells (Robb, 1985). These lymphoblast will proliferate in response to IL-2. Since CTLL are highly susceptible to mycoplasma contamination (Sinigaglia et al., 1985). Using contaminated cell lines reduce sensitivity, accuracy and reproducibility of IL-2 assays. Recently, it has been reported that interleukin 4 (IL-4), another T cell derived lymphokine, acting primarily on B cells (Howard et al., 1982), may cause proliferation of CTLL and also T lymphoblast (Severinson et al., 1987). IL-2 bioassay may, therefore, detect IL-4 in addition to IL-2. However, the amounts of IL-4 required to induce effective proliferation of either CTLL or lymphoblast are extremely high (Gearing et al., 1985; Severinson et al., 1987). Moreover, IL-4 had no measurable effect on T cells of the heterologous species (Mosmann et al., 1987). From above fact, we

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decided not to use CTLL but select short-term lectin-induced lymphoblast from Con A stimulated mouse spleen cells as target cells for assay of human IL-2 activity in order to eliminate IL-4 effect in our study.

In vitro IL-2 production of peripheral blood mononuclear cells (PBMC), many T cell mitogens have been tried to stimulate T cells. PHA is commonly used with human cells and Con A with murine cells (Kappler and Marrack, 1986). PHA induced IL-2 production in SLE patients was significantly less than normal populations (Alcocer-Varela et al., 1982; Miyasaka et al., 1984). However, IL-2 production in SLE patients and normal subjects were not significantly different when using combined mitogens such as PHA and PMA (Murakawa and Sakane, 1988) or Con A and PMA (Sibbitt et al., 1984; Draeger et al., 1986) stimulated PBMC. Thus, the PHA alone was selected in our study.

In titration of optimum condition for IL-2 production, it was found that 1.0 μg/ml PHA-P 1.0 x 10^6 PBMC/ml and 18 hours were PHA-P concentration, PBMC concentration, and incubation time for maximum IL-2 production respectively. Fetal calf serum (FCS) and human AB serum are frequently used in culture media for support the growth of human PBMC and increase in vitro IL-2 production (Alvarez et al., 1979). Recently, it was reported that FCS can stimulate resting human PBMC to acquire IL-2 receptors expression (Lakhanpal and Handwerger, 1986). By means of these receptors, IL-2 is able to

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enhance T cell proliferative response to mitogen and may raise IL-2 production in vitro (Ashman, 1984). To eliminate this effect, human AB serum was used to supplement the culture medium instead of FCS for IL-2 production in our study.

Concanavalin A (Con A; plant lectin) is one of the most potent mitogens for thymus-derived T lymphocytes. It has been shown that human T cells can be induced with Con A to be come cells which actively regulate immune function such as helper or suppressor cells, and some of which can express receptors for autologous erythrocytes. By using the autologous erythrocyte rosette technique, Con A-induced suppressor cells can be identified and separated from non-suppressor cells (Sakane et al., 1981). The suppressor cells confined to the autorosetting T cell populations, are not only $CD8^+$ marker but also $CD4^+$ marker (Takada et al., 1983).

In this study, the autologous erythrocyte rosette technique was used as suppressor T cells. Using this method, we got over 80% of AR^+ cells from the bottom of centrifuge tube. Suppressive function was assayed by the cell co-culture technique (Sakane et al., 1981). AR^+ cells of normal subjects and SLE patients were co-culture with autologous rested PBMC or heterologous rested normal PBMC for IL-2 suppressive study. We could not see suppressive effect of AR^+ cells when maximum dose of PHA-P (1.0 μg/ml) were used but we clearly noted this effect by using

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suboptimum dose of PHA-P (0.5 µg/ml). Therefore, this suboptimum dose of PHA-P were used in this study.

The titration of Con A concentration for preparation AR^+ cell was studied. It was found that suppressor T cells activity separated by autorosette formation is correlated with concentration of Con A (Shou et al., 1976). The suppressor function of AR^+ cells was confirmed by clearly decreased IL-2 production on increasing AR^+ cells to a constant rested PBMC. In order to differentiate the hyperfunction of suppressor T cells (AR^+ cells) of SLE patients from normal subjects, it is important to select the Con A concentration, to prepare AR^+ cells, which will not gave maximum suppression. Thus, we selected 5 µg/ml Con A to produce AR^+ cells to suppress IL-2 secretion in our SLE patients because this was the lowest concentration of Con A for AR^+ cells production.

Three possible mechanisms can decrease IL-2 activity inculture supernatants. These are needed to be concerned by all investigators : 1) the ability of AR^+ cells to absorb IL-2 in culture supernatants (Palacious and Moller, 1981), 2) the possibility of AR^+ cells to decrease PHA-P in culture supernatants, 3) crowding effect (Morimoto et al., 1982). In this study, we found that AR^+ cells had negligible capacity to absorb IL-2 in culture supernatants. This negligible absorption seen may be from a) small amount of IL-2 binding to IL-2 receptor on activated T cells b) IL-2 could not be internalized to the cells

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(Fujii et al., 1986). We did not find AR+ cells capable in decreasing PHA-P concentration in culture supernatants. These result may explained that AR+ cells, activated T cells, would diminish the number of surface mitogen receptor and rapidly increase surface IL-2 receptor expression (Meuer et al., 1984). Finally, no suppression of IL-2 secretion when AR- cells were added to rested PBMC instead of AR+ cells clearly indicated that this inhibition was not due to crowding effect.

IL-2 plays an important role in immunological reaction such as the generation of cytotoxic T cells (Wagner and Rollinghoff, 1978; Erad et al., 1985), the augmentation of natural killer cells activity (Henney et al., 1981), the generation of lymphokine-activated killer cells (Grimm et al., 1982), the proliferation and enhancement of immunoglobulin synthesis of B cells (Farrar et al., 1978 ; Ralph et al., 1984). Therefore, it is interesting to study IL-2 production in immunological disease such as SLE.

Systemic lupus erythematosus (SLE) is a disorder representative of autoimmune disease and characterized by polyclonal B cells activation (Decker et al., 1979; Kunkel, 1983). Hyperactive B cells are known to produce excessive amount of autoantibodies and other immunoglobulins (Decker et al., 1979; Fauci and Moutsopoulos et al., 1981; Saiki et al., 1985). Inspite of hyperfunction of B cells, dysfunction of T cells (Fauci et al., 1978; Sagawa et al., 1978; Delfraissy et al., 1980) and

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abnormalities of macrophage (Burns et al., 1982) were also found in SLE patients either from the disease itself or from drug therapy. In this study, IL-2 production was used as an indicator for CMI function. We expected to see low IL-2 production in these patients as observed by others (Alcocer-Varela et al., 1983; Linker-Israeli et al., 1983; Miyasaka et al., 1984; Murakawa et al., 1985; Huang et al 1986). Therefore, we plan to study whether the abnormal IL-2 production in these patients caused by a defect in the producer lymphocyte itself or by excessive suppression by suppressor T cells. This will be very useful to clearify the cellular basis for abnormal immunoregalation in SLE patients.

Twenty-two female SLE patients were selected by Dr. Prakong Vithayasai, Department of Medicine, Faculty of Medicine, Chiang Mai University. However, PBMC from nine SLE patients were not enough for assay IL-2 production and suppression and some of PBMC were found to be contaminated with neutrophils greather than 15% which significantly reduce IL-2 production (Grote et al., 1987). Therefore, we have only thirteen SLE patients for this study. All twelve SLE patients have not been on steroid prior to the study. Only one SLE patient has been treated with prednisolone doses 50 mg/day for 7 months but her blood was drawn after steroid was discontinued for 24 hours. It was demonstrated that corticosteroid can inhibit IL-2 production by lymphocyte (Crabtree et al., 1979;

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Gillis et al., 1979a) and return to normal within 24 hours (Bishop et al., 1968). All cases are active SLE (stage 1-3).

In vitro IL-2 production of freshly isolated PBMC were done in thirteen SLE patients and thirteen normal subjects. By using two - tailed student't test, the mean of IL-2 production from SLE patients were significantly less than those of normal subjects. This was similar to previous reports in human SLE (Linker-Israeli et al., 1983 ; Miyasaka et al., 1984 ; Murakawa et al., 1985 ; Huang et al., 1986) and in 3 stain of inbred mice with SLE - like disease (Dixon, 1985 ; Theofilopoulos and Dixon, 1985). This defect precedes the clinical development of the disease, suggesting that in SLE - prone mice, the deficiency in IL-2 production might contribute to the disease development (Dauphinee et al., 1981). Therefore, the decreased IL-2 production in human could be contributed to the development of autoimmune disease. The decreased IL-2 production in our SLE cases are not correlated with disease activity and duration. This is similar to previous studies (Miyasaka et al., 1984; Murakawa et al., 1985). This indicates that this deficit in SLE patients is unlikely to be the result of the disease severity.

Some investigator reported that the diminished IL-2 production in SLE patients was restored when T cells were rested for 2 to 3 days in culture medium before stimulation with mitogen (Huang et al., 1986). However, other recent studies showed that IL-2 defect was not normalized by resting the PBMC of SLE patients for 3

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days (Mc Kenna et al., 1988). In our study, PBMC of all SLE patients and normal subjects were rested for 2 days (48 hours) before stimulating with PHA-P. The mean of IL-2 production by freshly isolated and rested PBMC were not significantly different. The IL-2 production in the SLE patients is repeated demonstrated to be significant lower than normal controls and the IL-2 production did not increase after resting. Therefore, decrease IL-2 production in our SLE cases were not due to exhausted T cells. The differences of Mc Kenna et al's and our from Huang et al's result (1986) can be explained by different methodology. We and Mc Kenna et al used PBMC stimulated with PHA but Huang et al used T cells stimulated with combined mitogen (PHA and PMA).

Decreased IL-2 production in SLE patients may be from suppressive activity of suppressor T cells (Linker-Israeli et al., 1985). This was proved by removal of $CD8^+$ cells restored the IL-2 secretion of $CD4^+$ cells and adding back autologous $CD8^+$ cells caused IL-2 hyposecretion. However, it was reported that IL-2 hyposecretion in SLE patients was not due to active suppressor cells (Huang et al., 1986). He and his coworker added fresh $CD8^+$ cells to rested $CD4^+$ cells did not lead to excessive suppression of IL-2 secretion. Since suppressor T cells separated by phenotype expression ($CD8^+$ cells) could not covered all suppressor T cells population (Hirohata et al., 1989). It has been reported that suppressor T cells separated by using autologous erythrocyte rosette technique could

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represent all suppressor T cells population (both $CD4^+$ and $CD8^+$ cells) (Sakane et al., 1981 ; Takada et al., 1983). Thus, in our study, suppressor T cells were separated by autologous erythrocyte rosette technique and suppressor T cells received from this method will be selected to suppress IL-2 secretion. The mean of percentage of suppression of IL-2 secretion by AR^+ cells of SLE patients co-culture with autologous rested PBMC were not significantly different from those of AR^+ cells of normal subjects co-culture with autologous rested PBMC. These results are consistent with Huang et al's study (1986). It should be noted here that Huang et al separated suppressor T cells by monoclonal antibodies (CD 4 and CD 8), but we separated suppressor T cells by autologous erythrocyte rosette technique which covered all $CD4^+$ and $CD8^+$ suppressor effector cells (Takada et al., 1983). Thus, it seem that the circulating blood of SLE patients contains a population of T cells that have normal suppressor potential. However, Linker-Israeli et al (1985) reported that the inhibition of IL-2 production was over active suppressor T cells ($CD8^+$ cells). The differences of Huang et al and our result from Linker-Israeli et al can be explained by methodological difference ; Huang et al and our used suppressor T cells co-culture with rested cells (T cells or PBMC), but Linker - Israeli et al used suppressor T cells co-culture with fresh T cells.

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Linker-Israeli et al revealed that although there was autologous suppression as decribed before, $CD8^+$ cells from SLE patients did not suppress IL-2 secretion by normal CD8-depleted cells (heterologous suppression) and $CD8^+$ cells from normal subjects did not suppress IL-2 secretion by either autologous or heterologous CD8-depleted cells. This may indicated that resting $CD8^+$ cells could not suppress IL-2 secretion by CD8 - depleted cells except the $CD8^+$ cells were prior activated. In this study, we tested the ability of AR^+ cells of SLE patients to suppress IL-2 secretion from normal rested PBMC (heterologous suppression). The mean percentage of IL-2 suppression by AR^+ cells of SLE patients co-culture with normal rested PBMC were not significantly different from those of AR^+ cells of normal subjects co-culture with heterologous rested normal PBMC. Thus, our resuts indicated that AR^+ cells of SLE patients and normal subjects could equally suppress IL-2 secretion by heterologous rested normal PBMC. The differences of our result from Linker-Israeli et al can be explained by methodological difference as previously described.

We demonstrated that SLE patients had statistically significant decreased percentage of circulating T cell subsets bearing the autologous erythrocyte rosette markers (AR^+ cells) when compared with normal subjects. The decreased percentage of circulating AR^+ cells in SLE patients were not correlated with disease

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activity (r = -0.24), disease duration (r = -0.13), and IL-2 production by freshly isolated PBMC of SLE patients (r = 0.57). These results supported our finding that the decreased IL-2 production was due neither to excessive amount of supressor T cells nor to hyperfunction of suppressor T cells. It has been reported that active SLE patients who were not receiving corticosteroid therapy were T lymphopenic due to a marked reduction of the $CD4^+$ cell subset (Bakke et al., 1983 ; Stohl and Singer, 1987). $CD4^+$ cells are believed to be the main IL-2 producting lymphocyte subset (Moretta, 1985). Recently, it was found that IL-2 is produced mainly by the $CD4^+$ $2H4^+$ suppressor/inducer subset (Salmon et al., 1988). A specific loss of this subset has been shown to occur in active SLE patients (Morimoto et al., 1987 ; Sato et al., 1987 ; Raziuddin et al., 1989 ; Takada et al., 1989). The reduction of these cells may be the cause of decreased IL-2 production in SLE patients. Thus, further studies are needed to investigate whether a deficiency of $CD4^+$ $2H4^+$ suppressor/inducer subset contributes to the deficient in vitro IL-2 production in our patients population.

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