

Suppressive effect of Chinese medicinal herb, *Acanthopanax gracilistylus*, extract on human lymphocytes *in vitro*

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SUMMARY

We studied the effect of a Chinese medicinal herb, *Acanthopanax gracilistylus*, extract (AGE), on human lymphocytes *in vitro*. AGE markedly suppressed the proliferative responses of human peripheral blood lymphocytes stimulated with mitogens concanavalin A (Con A) and *Staphylococcus aureus* Cowan I (SAC). Both T cell and B cell activities—production of interferon-gamma and immunoglobulin—were suppressed by AGE. The mechanism of AGE-induced suppression of lymphocytes is to arrest the cell cycle at the G₀/G₁ stage without a direct cytotoxic effect. AGE also suppressed the alloantigen-specific cytotoxic T lymphocyte response. However, natural killer cell activity was less sensitive to the suppressive activity of AGE. In contrast, AGE markedly enhanced monocyte function to produce cytokines. These activities of AGE were associated with a 60-kD protein which was sensitive to treatment with pronase E, but not with NaIO₄. These results suggest that AGE has an immunomodulating activity on human lymphocytes and its properties could be clinically applied in the treatment of several diseases such as autoimmune and allergic diseases.

Keywords Chinese medicinal herb T cell B cell monocyte natural killer cell

INTRODUCTION

For a long time, Chinese medicinal herbs (CMH) have been traditionally used to treat several diseases such as autoimmune diseases and cancers in China [1]. Animal experiments and modern clinical trails have shown that many of these herbs have immunomodulating activities [2]. However, the mechanism of their effectiveness and the chemical nature of CMH have not been fully analysed. In a previous paper we studied the effect of several CMH on immune responses using human and mouse lymphocytes *in vitro* [3,4]. Some CMH such as *Oldenlandia diffusa* and *Cinamomum cassia presl* exhibit potentiating activity of lymphocyte functions. However, *Acanthopanax gracilistylus* extract (AGE) had a suppressive activity on lymphocytes. AG belongs to the *Acanthopanax* genus and has been used to treat patients with rheumatism. It has been reported that AG has an anti-inflammatory effect [5]. However, little is known about its mechanism and chemical nature. In this study we examined the effect of AGE on human lymphocytes and their chemical nature *in vitro*.

MATERIALS AND METHODS

Preparation of AGE

Dried barks of AG were purchased from the Chinese Herbal Medicine Co. (Shijiazhuang, Hebei, China). One gram of AG was steeped in 100 ml of distilled water at room temperature overnight, then boiled for 60 min. The extract was filtrated through a filter paper (Whatman type 42; Whatman International Ltd, Maidstone, UK) to remove insoluble materials and used for experiments as a crude sample after Millipore (Millipore, 0.22 µm pore size; Nippon Millipore Ltd, Tokyo, Japan) filtration.

Preparation of peripheral blood lymphocytes and in vitro culture

Peripheral blood lymphocytes (PBL) were isolated from the peripheral blood of healthy volunteers by density gradient centrifugation with a lymphoprep (Nycomed Pharm As, Oslo, Norway) at 400g for 30 min. PBL (2 × 10⁵) were cultured with several concentrations of AGE, 10 µg/ml concanavalin A (Con A; EY Labs, San Mateo, CA), 0.01% *Staphylococcus aureus* Cowan I (SAC; Funakoshi Yakuhin Co., Tokyo, Japan) in 0.2 ml of RPMI 1640 (Nissui Seiyaku Co., Yokohama, Japan) medium containing 10% fetal calf serum (FCS; Gibco, Grand Island, NY) in wells of flat-bottomed microtitre culture plates (Falcon no. 3072; Becton Dickinson Co., Lincoln Park, NJ) at 37°C for 3 days in 5% CO₂ and 95% air. The cells were labelled with 0.5 µCi of tritiated

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thymidine ($^3\text{H-TdR}$; specific activity 6.0 Ci/mmol; Amersham Plc, Aylesbury, UK) for the last 15 h and were harvested with the aid of a semiautomated cell harvester (Abe Kagaku Co., Chiba, Japan) [6]. The amount of radioactivity incorporated into the DNA in the cells was measured with a liquid scintillation counter (Aloka Co., Tokyo, Japan). The results are expressed as the mean *ct/min* of $^3\text{H-TdR}$ incorporated by the PBL with standard error (s.e.m.) in triplicate cultures.

B cell- and T cell-enriched fractions were prepared by a rosette formation method. PBL (10^7) were incubated with 10^9 sheep erythrocytes at 37°C for 15 min and at 4°C for 2 h and fractionated by density gradient centrifugation on a lymphoprep at 400 *g* for 30 min [7]. The sheep erythrocyte rosette-positive fraction (T cells) contained >90% CD3⁺ cells and <5% CD20⁺ cells. The sheep erythrocyte rosette-negative fraction (B cells) contained >90% CD20⁺ cells and <5% CD3⁺ cells by flow cytometric analysis using fluorescent antibodies.

Production and assay of interferon-gamma

PBL (10^6 /ml) were cultured with 10 $\mu\text{g/ml}$ Con A in the presence or absence of AGE in RPMI–10% FCS medium in culture dishes (Falcon no. 3002) at 37°C for 24 h and the culture supernatants were harvested. The amount of interferon-gamma (IFN- γ) in the culture supernatants was assayed by ELISA. The wells of flat-bottomed microtitre plates (Sumitomo Bakelite Co., Tokyo, Japan) were coated with anti-IFN- γ capture antibody (Pharmingen, Becton Dickinson Co., San Diego, CA), and the culture supernatant from PBL was added to the plate. After washing, biotin-labelled detection antibody, streptavidin-conjugated alkaline phosphatase (Pharmingen) and *p*-nitrophenyl phosphate (Zymed Labs Inc., San Francisco, CA) as a substrate were added. The optical density (OD) at 490 nm was measured using Immunoreader (Japan Intermed Co., Tokyo, Japan). The results were expressed as the pg of IFN- γ produced by 10^6 PBL and s.e.m. in triplicate cultures compared with recombinant human IFN- γ (Pharmingen) as the standard.

Production and assay of immunoglobulin

PBL (5×10^5 /ml) were cultured with 0.01% SAC in the presence or absence of AGE for 5 days. The culture supernatants were harvested, and total IgG in the culture supernatants was assayed by ELISA by the same method as for the detection of IFN- γ [8] using sheep anti-human IgG antibody, peroxidase-conjugated sheep anti-human IgG antibody (The Binding Site, Birmingham, UK) and *o*-phenylenediamine dihydrochloride (5.5×10^{-4} M; Wako Junyaku Co., Kyoto, Japan) in 0.01% H₂O₂ solution as a substrate. Purified human IgG was used as the standard. The results are expressed as the mean μg of IgG produced by 5×10^5 PBL with s.e.m. in triplicate cultures.

Cell cycle analysis

PBL (10^6) were cultured with 10 $\mu\text{g/ml}$ Con A in the presence or absence of AGE in RPMI–10% FCS medium in culture dishes (Falcon no. 3002) at 37°C for 3 days. Cells were harvested and washed with PBS pH 7.4, then fixed with 99.5% ethanol at 4°C for 2 h. Cells were treated with 0.25 mg/ml of RNase A (Sigma Chemical Co., St Louis, MO) at 37°C for 1 h. After washing, cells were stained with 500 $\mu\text{g/ml}$ propidium iodide (PI; Sigma) at room temperature for 10 min. Analysis was performed on a flow cytometer (Coulter Co., Hialeah, FL) [9].

The percentage of cells in each stage of the cell cycle was determined using a Cellfit analysis program on the staining profile of viable cells.

Induction and assay system of cytotoxic T lymphocytes

To induce CTL, PBL (2.5×10^6) were cultured with a mitomycin C (50 $\mu\text{g/ml}$; Kyowahakko Kogyo Co., Tokyo, Japan)-treated adult T cell leukaemia cell line, MT-2 (10^5), in 24-well culture plates (Falcon no. 3047) in 2 ml of RPMI–10% FCS medium at 37°C for 5 days in 5% CO₂ and 95% air [10]. The cytotoxic T lymphocyte (CTL) activity was detected by culturing *in vitro* sensitized PBL (10^5) and ^{51}Cr -sodium chromate (1.2 Ci/mg; New England Nuclear, Boston, MA)-labelled MT-2 cells (10^4) in wells of round-bottomed microtitre culture plates (Falcon no. 3077). After effector-to-target cell interaction at 37°C for 5 h, the supernatant in each well was harvested and the radioactivity in the supernatant was counted with a gamma counter (Aloka Co.) [11]. The percentage of specific lysis was calculated from the formula: % specific lysis = [(experimental release – control release)/(maximum release – control release)] \times 100, where maximum release was obtained by incubating ^{51}Cr -labelled MT-2 cells in the presence of 1% Nonidet P-40 (Sigma), and control release was obtained by incubating MT-2 cells with unsensitized PBL. The results were expressed as the means and s.e.m. of the specific lysis of target cells in triplicate cultures.

Assay system of natural killer cell activity

The assay system of natural killer (NK) cell activity was the same as the assay system of CTL activity, except for the use of PBL without 5 days culture as effector cells, and U937 cells as target cells.

Production and assay of tumour necrosis factor

PBL (10^6 /ml) were cultured in RPMI–10% FCS medium in culture dishes (Falcon no. 3002) at 37°C for 2 h. Non-adherent cells were discarded by gentle agitation and dish-adherent cells were obtained by scraping with a rubber policeman. Adherent cells (5×10^5 /ml) were stimulated with 10 $\mu\text{g/ml}$ lipopolysaccharide (LPS) from *Escherichia coli* (Sigma) in the presence or absence of AGE at 37°C for 18 h and the culture supernatants were harvested. The activity of tumour necrosis factor (TNF) in the culture supernatants was assayed according to the method of Ruff & Gifford [12]. L929 cells (10^4) were cultured in RPMI–10% FCS medium in wells of flat-bottomed microtitre culture plates (Falcon no. 3072) for 20 h. The culture medium was then replaced with TNF samples and the cells were further cultured for 20 h in the presence of 1 $\mu\text{g/ml}$ actinomycin D (Sigma). After culture, the cells were stained with 0.2% crystal violet (Sigma) in 20% methanol, were solubilized by 1% SDS (Sigma) and the OD at 490 nm was measured by Immunoreader. The results were expressed as the units of TNF produced by 5×10^5 monocytes and s.e.m. in triplicate cultures, compared with recombinant human TNF- α (Pharmingen) as the standard.

Fractionation of AGE by gel filtration

Crude AGE was applied to a Sephacryl S-200 column (1.9 \times 45 cm; Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated and eluted with PBS. Aliquots of 2 ml were collected. The sugar and protein content in each fraction was measured according to the phenol-sulphuric acid method [13] and OD at 280 nm, respectively. The activity of each fraction was assayed by the inhibition of PBL

Table 1. Effect of *Acanthopanax gracilistylus* extract (AGE) on the proliferative response of peripheral blood lymphocytes† (PBL)

| PBL cultured with | ³ H-TdR uptake by PBL (ct/min) | | |
|-------------------|---|------------|------------|
| | Case 1 | Case 2 | Case 3 |
| (-) | 217 ± 6 | 166 ± 26 | 141 ± 3 |
| Con A | 8377 ± 65 | 8681 ± 789 | 3967 ± 225 |
| SAC | 1586 ± 136 | 1077 ± 100 | 896 ± 28 |
| AGE‡ | 156 ± 10* | 129 ± 15* | 86 ± 11* |
| Con A + AGE | 125 ± 3* | 129 ± 10* | 102 ± 6* |
| SAC + AGE | 109 ± 4* | 139 ± 7* | 101 ± 3* |

†PBL (2×10^5) were cultured with 10 µg/ml concanavalin A (Con A), 0.01% *Staphylococcus aureus* Cowan I (SAC) or 10 µg/ml AGE for 3 days, labelled with ³H-TdR for the last 15 h and harvested. Then the ³H-TdR uptake by PBL was counted. Results are expressed as mean ct/min of ³H-TdR uptake with s.e.m. in triplicate cultures.

‡AGE (1 g dried weight) was extracted in 100 ml of boiling water for 1 h. The extract contained 1780 µg/ml protein determined by the Lowry method.

*Significantly inhibited.

proliferation. The active fractions were combined and further purified in a Sephadex G-100 column (Pharmacia) and the resulting active fractions were combined and used as the purified component.

SDS-PAGE and elution of samples from SDS-PAGE

Crude and fractionated samples of AGE were separated with SDS-PAGE [14] using 10% polyacrylamide (Funakoshi Yakuhin Co.). The gel was stained with coomassie brilliant blue (CBB; Sigma).

The protein was eluted from the gel slices using an Electro-eluter (BioRad Labs, Hercules, CA) at 10 mA/glass tube for 6 h. The eluted protein was recovered and dialysed against PBS.

Treatment with pronase E and NaIO₄

Crude and fraction samples of AGE were incubated with 0.4 mg of pronase E (Serva Feinbiochemica GmbH & Co., Heidelberg, Germany) at 30°C in 4 ml of 0.1 M Tris-HCl buffer (pH 8.0) containing 50 mM CaCl₂. After 36 h incubation, 0.2 mg of pronase E was supplemented and the incubation was continued for another 36 h. The reaction mixture was then heated at 100°C for 10 min to inactivate the pronase E and dialysed against PBS.

For NaIO₄ treatment, crude and fraction samples of AGE were incubated in 100 µl of 0.1 M NaIO₄ (Sigma) at 25°C for 4 h. Then 250 µl of 20% ethylene glycol (Sigma) were added [15] and the samples were dialysed against PBS.

Statistical analysis

All experiments were repeated at least three times and some representative results are shown in the tables and figures. Statistical analysis was performed by Student's *t*-test. A confidence level of <0.05 was considered significant [16].

RESULTS

Inhibitory effect of AGE on PBL proliferative responses

At first we studied the effect of AGE on the proliferative responses

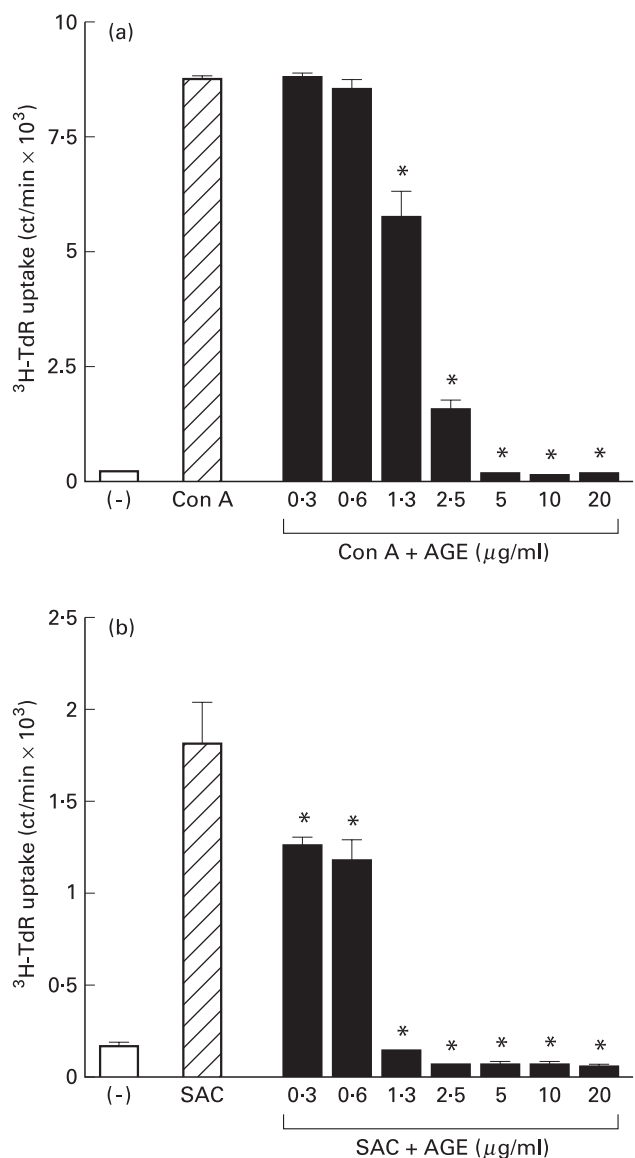


Fig. 1. Dose-response curve of *Acanthopanax gracilistylus* extract (AGE). Peripheral blood lymphocytes (PBL; 2×10^5 /well) were cultured with concanavalin A (Con A) (a) or *Staphylococcus aureus* Cowan I (SAC) (b) in the presence of several concentrations of AGE at 37°C for 3 days. Results are expressed as mean ct/min of ³H-TdR uptake with s.e.m. in triplicate cultures.*Significantly inhibited.

of PBL. As shown in Table 1, AGE markedly inhibited the proliferative responses of PBL stimulated with Con A and SAC. Figure 1 shows the dose-response curve of the inhibitory effect of AGE on Con A (Fig. 1a) and SAC (Fig. 1b) responses. The responses of sheep erythrocyte rosette-positive cells (T cells) and sheep erythrocyte rosette-negative cells (B cells) were similarly inhibited by AGE (Fig. 2), suggesting that AGE directly affects both T and B cells.

AGE inhibited IFN-γ and IgG production by PBL

We next studied the effect of AGE on IFN-γ and IgG production by PBL as a representative function of T cells and B cells,

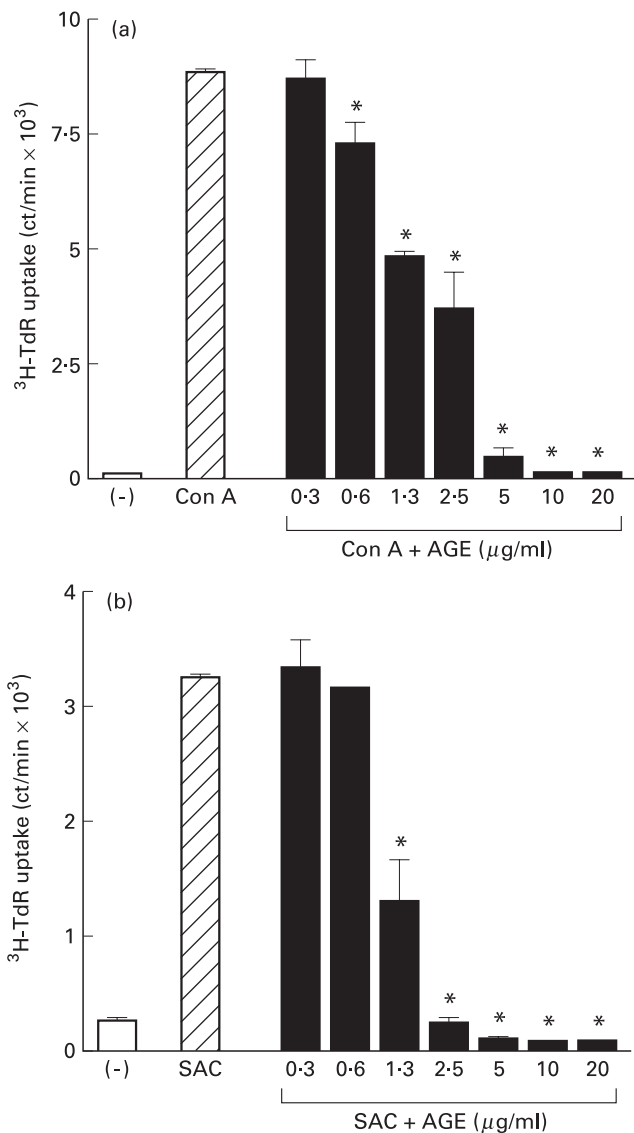


Fig. 2. Inhibitory effect of *Acanthopanax gracilistylus* extract (AGE) on B cell- and T cell-enriched fractions. Peripheral blood lymphocytes (PBL) were fractionated with sheep erythrocytes. Sheep erythrocyte rosette-positive cells (T cells) and sheep erythrocyte rosette-negative cells (B cells) were cultured with concanavalin A (Con A) (a) or *Staphylococcus aureus* Cowan I (SAC) (b) in the presence of AGE at 37°C for 3 days. Results are expressed as mean ct/min of ³H-TdR uptake with s.e.m. in triplicate cultures. *Significantly inhibited.

respectively. AGE suppressed both IFN- γ production in Con A-stimulated PBL (Fig. 3a) and IgG production in SAC-stimulated PBL (Fig. 3b) in a dose-dependent manner.

Effect of AGE on the cell cycle

To study the mechanism of AGE-induced suppression of PBL responses, we examined the cell cycle by flow cytometry. PBL were stimulated with Con A in the presence or absence of AGE for 72 h, washed, stained with PI and the cell cycle was analysed. In control cells without stimulation, 3% of cells were in the S or G₂ phase and 90% of cells were in the G₀/G₁ phase of the cell cycle (Fig. 4). Con A stimulation induced 15% of cells to move to the S

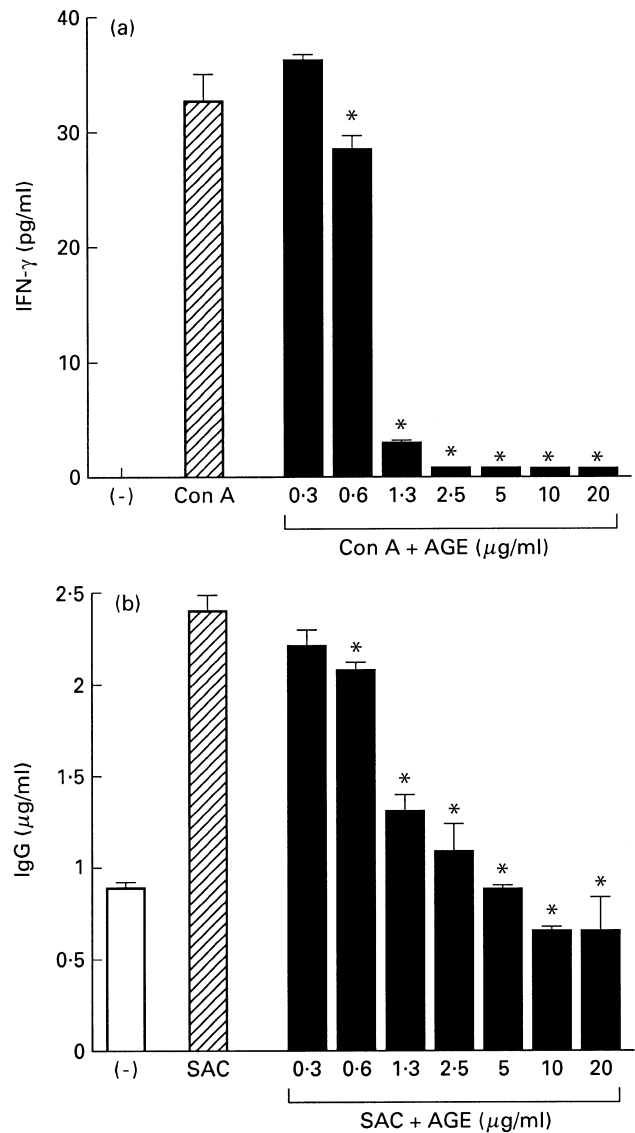


Fig. 3. Inhibitory effect of *Acanthopanax gracilistylus* extract (AGE) on IFN- γ and IgG production by peripheral blood lymphocytes (PBL). PBL (1×10^6) were cultured with concanavalin A (Con A) in the presence of several concentrations of AGE for 24 h. The amount of IFN- γ in the culture supernatants was assayed by ELISA. Results are expressed as mean pg of IFN- γ produced by 10^6 PBL with s.e.m. in triplicate cultures (a). PBL (5×10^5) were cultured with *Staphylococcus aureus* Cowan I (SAC) in the presence of several concentrations of AGE for 5 days. The amount of IgG in the culture supernatants was examined by ELISA. Results are expressed as mean μ g of IgG produced by 5×10^5 PBL with s.e.m. in triplicate cultures (b). *Significantly inhibited.

or G₂ phase and the cells in the G₀/G₁ phase decreased to 80%. After AGE treatment, the cell cycle remained at the level of the control cells and necrotic and apoptotic cells did not increase. Furthermore, the viability of AGE-treated PBL was not different from that of non-treated PBL (data not shown). These results suggest that AGE inhibits cell cycle progression, but does not induce cell death.

AGE inhibited CTL response, but not NK cell activity

Alloantigen-specific CTL was induced by culturing PBL with

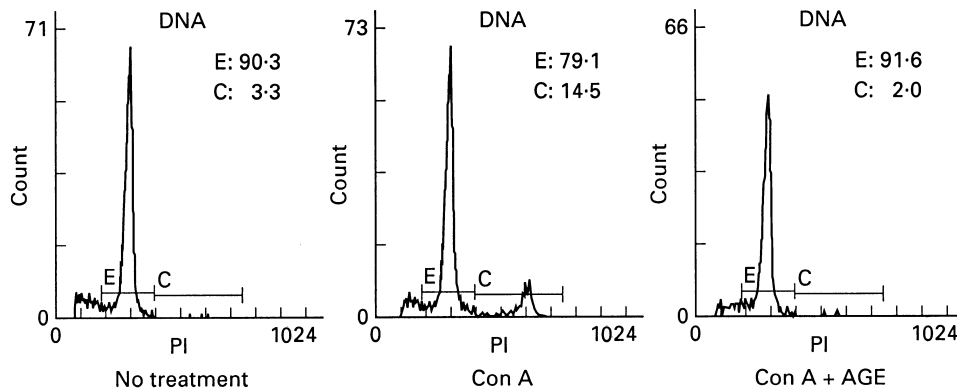
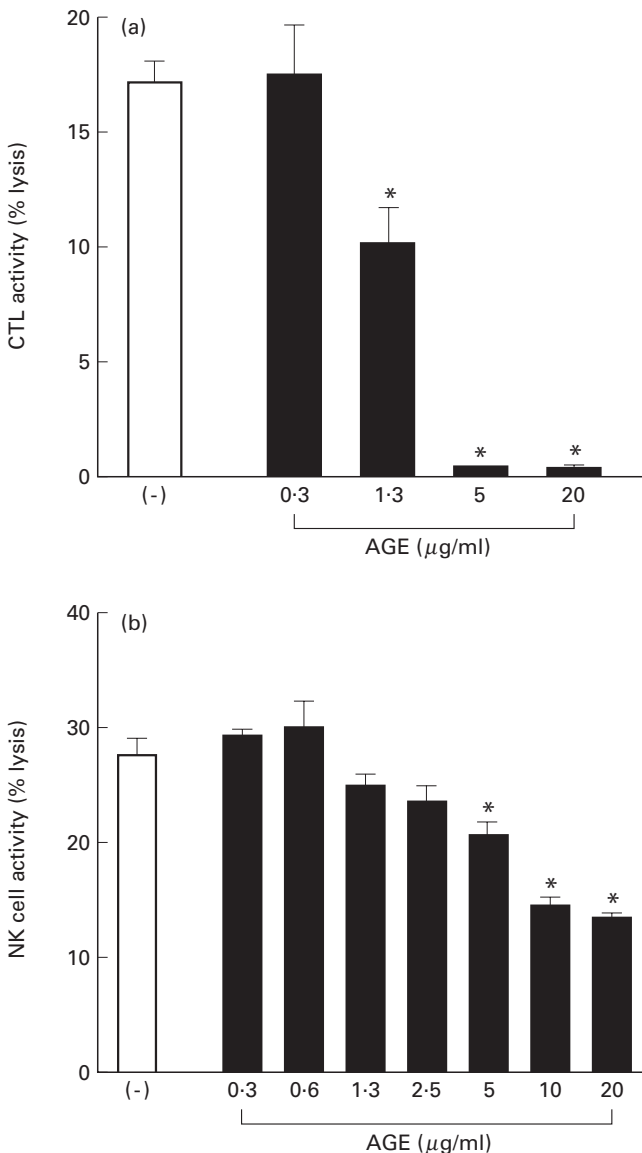


Fig. 4. Effect of *Acanthopanax gracilistylus* extract (AGE) on the cell cycle. Peripheral blood lymphocytes (PBL; 2×10^6) were stimulated with concanavalin A (Con A) in the presence or absence of AGE for 3 days, stained with propidium iodide (PI) and analysed using a flow cytometer. The results are expressed as the profile of PI⁺ cells and the percentage of each cell cycle. E, G₀/G₁; C, S/G₂.



MT-2 cells for 5 days and the activity was assayed with ⁵¹Cr-labelled MT-2 cells. AGE suppressed the induction of CTL in a dose-dependent manner (Fig. 5a).

The effect of AGE on NK cell activity was determined by incubating PBL with AGE for 24 h, and the activity was assayed using ⁵¹Cr-labelled U937 cells as target. NK cell activity was less sensitive to AGE (Fig. 5b).

AGE enhanced cytokine production by monocytes

We next examined the effect of AGE on monocyte function by studying cytokine production. As shown in Fig. 6, AGE markedly stimulated monocytes to produce TNF in a dose-dependent manner. AGE also enhanced LPS-induced TNF production. Other cytokine production by monocytes such as IL-1α and IL-6 was also induced by AGE (data not shown).

Active components of AGE are proteins

We studied the chemical nature of the active component of AGE. At first, AGE was chromatographed on a Sephacryl S-200 column. The activity of each fraction was assayed by the inhibition of PBL proliferative responses (Fig. 7a). The active fractions were combined, lyophilized, dialysed and rechromatographed on a Sephadex G-100 column (Fig. 7b). As illustrated in Fig. 7, the active component was eluted as a single peak whose molecular weight was about 50 kD.

Next, we treated crude and fraction samples of AGE with pronase E and NaIO₄. As shown in Fig. 8, treatment with pronase E significantly abrogated its activity, but treatment with NaIO₄ did

Fig. 5. Effect of *Acanthopanax gracilistylus* extract (AGE) on cytotoxic T lymphocytes (CTL) and natural killer (NK) cell activity. Peripheral blood lymphocytes (PBL; 2.5×10^6) were cultured with mitomycin C-treated MT-2 cells (10^5) in the presence of several concentrations of AGE at 37°C for 5 days to induce CTL. Cytotoxic activity was detected by ⁵¹Cr-labelled MT-2 cells as the target cells. The results are expressed as mean and s.e.m. of the percentage of specific lysis of the target cells in triplicate cultures (a). PBL (5×10^5) were precultured with various concentrations of AGE for 24 h, then cultured with ⁵¹Cr-labelled U937 cells as the target for 5 h. The results are expressed as the mean percentage of specific lysis of U937 cells and s.e.m. in triplicate cultures (b). *Significantly suppressed.

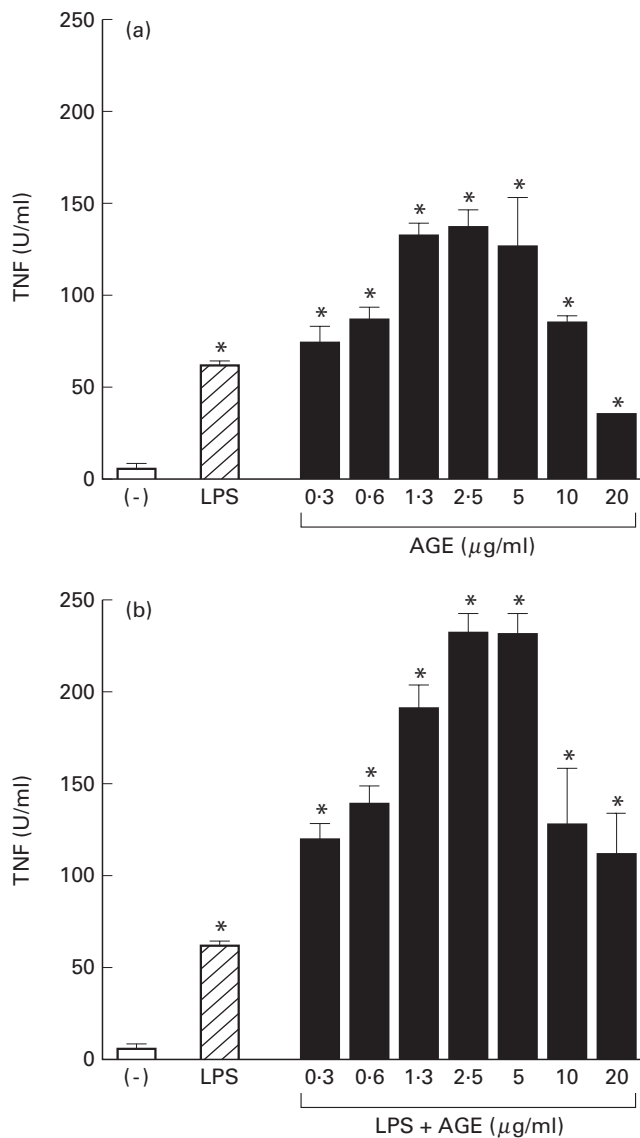


Fig. 6. Effect of *Acanthopanax gracilistylus* extract (AGE) on tumour necrosis factor (TNF) production by monocytes. Peripheral blood lymphocytes (PBL; 1×10^6) were cultured at 37°C for 2 h. Non-adherent cells were discarded. Adherent cells (5×10^5) were cultured with 10 µg/ml lipopolysaccharide (LPS) in the presence or absence of AGE for 24 h. TNF activity in the culture supernatants was assayed using L929 cells. The results are expressed as units of TNF produced by 5×10^5 monocytes and s.e.m. in triplicate cultures. *Significantly enhanced.

not, suggesting that the active components of AGE are proteins but not sugars.

We also purified the active component of AGE by SDS-PAGE. Crude AGE showed two major bands of approx. 50–70 kD mol. wt. The column-purified fraction showed one band at approx. 60 kD mol. wt. The eluted fraction of this band (F2) showed inhibitory activity on the proliferative response of PBL (Fig. 9).

DISCUSSION

CMH have been traditionally used to prevent and treat numerous types of diseases in China, especially autoimmune diseases and

cancers [1]. AG has been used to treat patients with rheumatism and is reported to have an anti-inflammatory effects [5]. However, its mechanism of action and chemical nature have not been fully analysed. In this study, we examined the effect of AGE on human lymphocytes *in vitro*.

AGE markedly inhibited the proliferative responses of PBL stimulated with mitogens, such as Con A and SAC, in a concentration-dependent manner. AGE not only inhibited the proliferative responses of T cells and B cells, but also their functions of producing IFN- γ and immunoglobulin. The suppressed B cell responses with AGE were not due to the suppressed T cell responses, because the proliferative responses and immunoglobulin production by purified B cells and cell lines maintained *in vitro* were similarly suppressed by AGE as well as PBL (data not shown). The mechanism of AGE-induced immunosuppression is to arrest the cell cycle at the G₀/G₁ stage without a direct cytotoxic effect on lymphocytes. AGE also inhibited the induction of alloantigen-specific CTL, but NK cell activity was less sensitive to AGE. In contrast, AGE stimulated monocytes to produce cytokines such as TNF, IL-1 and IL-6. The enhancing effect of cytokine production with AGE was not due to a cytotoxic effect on monocytes, which can release intracellular cytokines, because there was no significant difference between the viability of AGE-treated and untreated monocytes. The suppressed T cell responses with AGE were not due to non-specific monocyte activation, which can produce a large amount of non-specific suppressive factors [17], because the proliferative responses and IFN- γ production by purified T cells and cell lines maintained *in vitro* were also suppressed by AGE as well as PBL (data not shown). Thus, AGE is not a non-specific immunosuppressant, but its activity has cellular selectivity. The mechanisms of the different susceptibilities to AGE between T cells, B cells and monocytes are under investigation.

Several proteins or polysaccharides originating from higher plants have immunopotentiating and anti-tumour activities as biological response modifiers [18–21]. *Acanthopanax senticosus*, as well as its polysaccharide fraction, enhanced the phagocytic activity of macrophages and inhibited the growth of transplanted tumours in mice [2,22,23]. We also studied the effect of AGE on the growth of tumour cells *in vitro*. AGE inhibited the growth of tumour cells in a dose-dependent manner. Furthermore, we found that monocytes treated with AGE had a growth-inhibitory effect on tumour cells. We think that monocytes/macrophages are activated by AGE and work suppressively on tumour cell growth (data not shown).

Next, we studied the chemical nature of AGE. We purified AGE by gel filtration chromatography. The active component of AGE was eluted as a single peak by sequential filtration using Sephacryl S-200 and Sephadex G-100 columns. All activities to suppress T and B cell functions, and to enhance monocyte functions, resided in this fraction. The activity is sensitive to treatment with pronase E, but not to NaIO₄, suggesting that the active component of AGE is a protein, but not a sugar. We further purified AGE using SDS-PAGE. The active component of AGE was fractionated around 60 kD by SDS-PAGE.

We did not extensively examine the molecular mechanism of AGE-induced immunosuppression in this study.

The next step to be elucidated in this series of studies is to clarify the nature of the binding molecules for AGE on the cells and intracellular events.

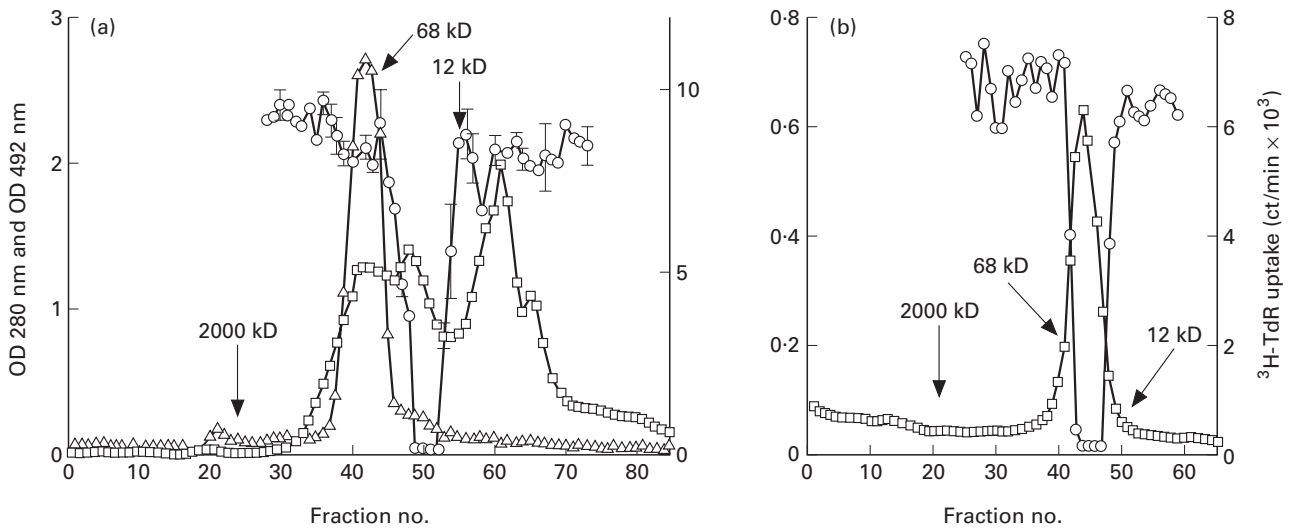


Fig. 7. Fractionation of active components of *Acanthopanax gracilistylus* extract (AGE). AGE was chromatographed on a Sephacryl S-200 column (a). The active fractions were combined and rechromatographed on a Sephadex G-100 column (b). The protein concentration was detected by optical density (OD) at 280 nm (□). The glucose concentration was measured using the phenol-sulphuric acid method (Δ). The activity of each fraction was determined by ³H-TdR uptake (○) of peripheral blood lymphocytes (PBL). The column size was determined by eluting marker proteins: void (blue dextran), bovine serum albumin (68 kD) and Cytochrome C (12 kD).

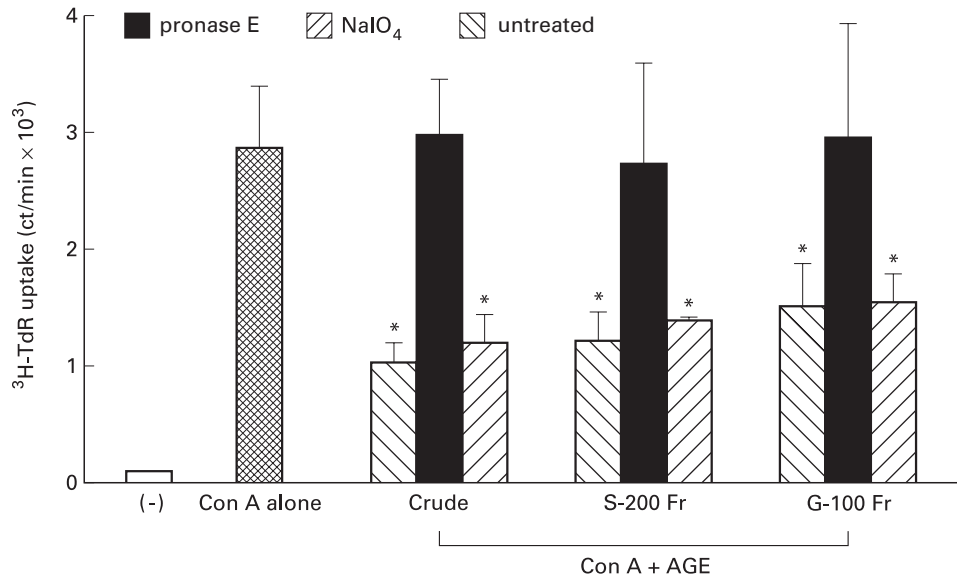


Fig. 8. Effect of pronase E or NaIO₄ treatment on *Acanthopanax gracilistylus* extract (AGE). Crude, S-200 and G-100 fractions of AGE were treated with pronase E, NaIO₄ or untreated and their activity was assayed by ³H-TdR uptake of peripheral blood lymphocytes (PBL) stimulated with concanavalin A (Con A). *Significantly suppressed.

As a preliminary experiment, we observed that AGE had a regulatory effect on retinoblastoma (Rb) protein and cyclin-dependent kinase (Cdk), which regulate the cell cycle (data not shown).

Although the study was performed only with an *in vitro* assay system, evidence that AGE has a selective immunomodulating activity on human lymphocytes provides a rational

basis for the efficacy of this medicinal herb. For example, AGE may be applied to the treatment of autoimmune and allergic diseases as an immunosuppressive drug and to the treatment of cancers as a cell cycle-regulating drug. Further purification of the active components and examination of their effects *in vivo* seem to be required for clinical applications.

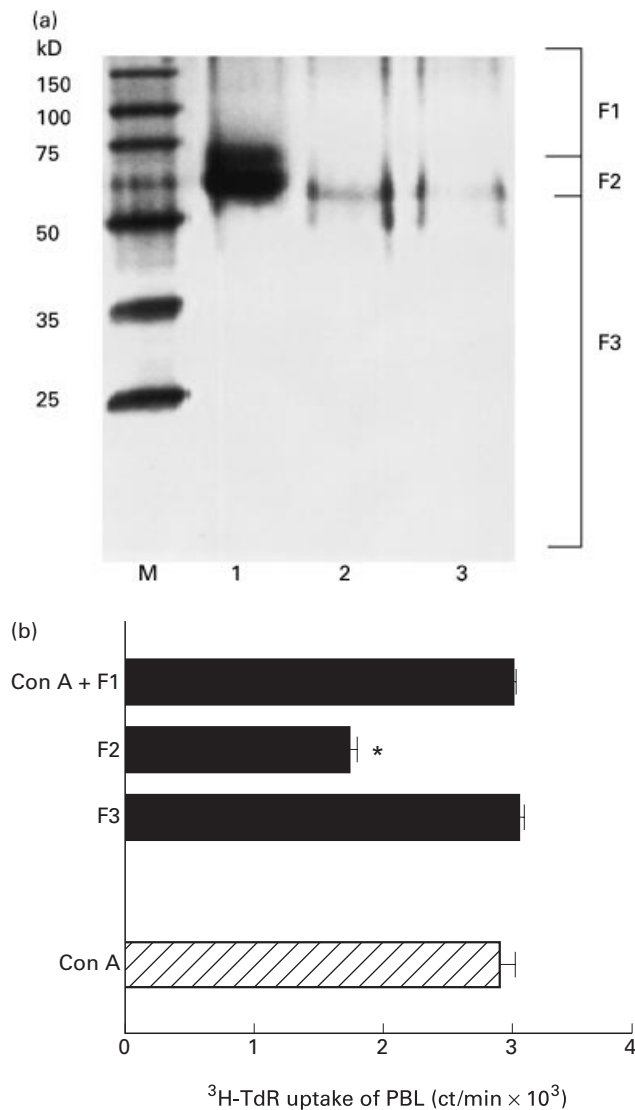


Fig. 9. Purification of *Acanthopanax gracilistylus* extract (AGE) with SDS-PAGE. Crude, S-200 and G-100 fractions of AGE were separated with SDS-PAGE and stained with coomassie brilliant blue (CBB) (a). Lane 1, crude AGE; lane 2, S-200 Fr; lane 3, G-100 Fr; M, molecular weight marker. SDS-PAGE was cut out into three fractions and the proteins in them were eluted. The activity of the elutions was assayed by the $^3\text{H-TdR}$ uptake of peripheral blood lymphocytes (b). *Significantly suppressed.

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