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Induction and separation of mouse helper T cells by lectins

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Summary. The capability of *Lens culinaris* agglutinin (LcA) to induce selectively the helper T cell activity affecting primary antibody response was demonstrated. In the presence of mouse spleen cells, activated with LcA at a concentration of 12·5 μg/ml, optimal augumentation of the humoral immune response to sheep erythrocytes (SRBC) was observed. It was also demonstrated that *Limulus polyphemus* agglutinin (LPA), which was shown to possess carbohydrate-binding specificity directed to sialic acid residues, preferentially agglutinated helper T cells. Conversely, peanut agglutinin (PNA), which binds preferentially to the sugar sequence β-D-Gal-(1→3)-D-GalNAc, did not agglutinate the helper cells. Furthermore, the stimulatory effect of LPA-agglutinated cells on the antibody response was abolished by treatment of the cells with anti-Thy-1·2 and complement. These results suggested that the helper cells induced by LcA were T cells and that they have abundant sialic acid residues exposed on the cell surface.

Abbreviations: BpA, *Bauhinia purpurea* agglutinin; C′, complement; Con A, concanavalin A; FACS, fluorescence-activated cell sorter; FCS, foetal calf serum; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; LcA, *Lens culinaris* agglunitinin; LPA, *Limulus polyphemus* agglutinin; 2-ME, 2-mercaptoethanol; PHA, phytohaemagglutinin P; PFC, plaque-forming cells; PNA, peanut agglutinin; PsA, *Pisum sativum* agglutinin; SEM, standard error of the mean; SRBC, sheep red blood cells; WfM, *Wistaria floribunda* mitogen; WGA, wheat germ agglutinin.

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INTRODUCTION

The differentiation and proliferation of B lymphocytes are regulated by functionally heterogeneous T lymphocytes such as helper, suppressor and memory T cells. These different T-cell subpopulations were shown to be separated by virtue of various cell surface markers present; such as Ly or Ia alloantigens (Cantor, Shen & Boyse, 1976; Okumura, Takemori, Tokuhisa & Tada, 1977). In addition, attempts were made to fractionate the heterogeneous cell population based on heterogeneity of the membrane-associated glycoproteins, by use of lectins having different carbohydrate binding specificities (Reisner, Linker-Israeli & Sharon, 1976; Haller, Gidlund, Hellström, Hammerström & Wigzell, 1978). Dutton (1972) reported that helper and suppressor T-cell activities could be induced when T lymphocytes were activated with mitogens such as concanavalin (Con A) or phytohaemagglutinin-P (PHA).

In a previous paper (Imai, Oguchi, Nakano, Sawada & Osawa, 1980), we reported that the mouse T cells which exert a suppressive effect on the primary antibody response of mouse spleen cells toward sheep red blood cells also stained strongly with peanut lectin, previously shown to recognize terminal sialic acid-free β-galactosyl residues on the cell surface (Terao, Irimura & Osawa, 1975; Pereira, Kabat, Lotan & Sharon, 1976).

In this paper, we describe the results of our attempts to induce preferentially different T-cell subsets by various lectins and to separate the induced helper T cells by use of lectins having different carbohydrate binding specificities.

MATERIALS AND METHODS

Mice

Eight to twelve-week-old female [C57Bl/6 × DBA/2]F_1 (BDF_1) hybrid mice were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan).

Media and reagents

RPMI-1640 was purchased from Grand Island Biological Co. (Grand Island, N.Y.), foetal calf serum (FCS) from Flow Laboratories (Stanmore, N.S.W., Australia), and N-2-hydroxyethyl-piperazine-N′-2-ethanesulphonic acid (HEPES) from Wako Pure Chemical Co. (Tokyo, Japan). The medium used was RPMI-1640 supplemented with 20 mM HEPES adjusted to pH 7·4 with NaOH, 4 mM glutamine, 1 mM pyruvate, 60 mg/l kanamycin (Meiji Seika Co., Tokyo, Japan), 5×10^{-5} M 2-mercaptoethanol (2-ME) and 5% FCS (RPMI-FCS), Eagle's minimum essential medium at pH 7·4 without phenol red [MEM(−)]. *Limulus polyphemus* agglutinin (LPA), peanut agglutinin (PNA), wheat germ agglutinin (WGA) and fluorescein-labelled LPA (FITC-LPA) were obtained from the E. Y. laboratory (San Mateo, CA) and PHA from the Difco Laboratories (Detroit, Mich). *Lens culinaris* agglutinin (LcA), *Bauhinia purpurea* agglutinin (BpA) and *Wistaria floribunda* mitogen (WfM) were prepared by the methods described previously (Toyoshima, Osawa & Tonomura, 1970; Osawa, Irimura & Kawaguchi, 1978; Toyoshima, Akiyama, Nakano, Tonomura & Osawa, 1974). Con A was purified by the method of Agrawal& Goldstein (1967) and *Pisum sativum* agglutinin (PsA) by the method of Onodera & Shinohara (1973).

In vitro *activation of mouse spleen cells with a lectin*

After decapitation of mice, spleens were removed and a spleen cell suspension (2×10^7 cells/ml) in RPMI-1640 (minus 2-ME) was prepared and incubated with various amounts of a lectin for 40 h at 37° in an atmosphere of 5% CO_2 and 95% air. After incubation, the spleen cells were purified by density gradient centrifugation over a mixture containing 5% Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden) and 15% Urografin (Schering, Berlin, West Germany) in RPMI-1640 (minus 2-ME) according to Kawaguchi, Matsumoto & Osawa (1974). The purified cells were then resuspended and washed three times with 0·1 M methyl α-D-mannopyranoside in the same medium. The cells were then finally resuspended in RPMI-1640 medium (minus 2-ME) and used as 'LcA-activated' cells.

Spleen cell cultures and plaque assay

2×10^6 normal spleen cells were cultured in RPMI-1640 (plus 2-ME) with $2–4 \times 10^5$ lectin-induced cells for assaying their function and 10^6 sheep red blood cells (SRBC) (Funabashi Farm Co., Chiba, Japan) in 0·1 ml of RPMI-1640-5% FCS at 37° in an atmosphere of 5% CO_2 and 95% air. Five days later, the number of direct IgM-producing plaque-forming cells (PFC) was determined according to the method of Cunningham & Szenberg (1968). Cultures were performed in triplicate.

Staining of 'LcA-activated' cells with FITC-LPA and separation on a fluorescence activated cell sorter (FACS II)

The 'LcA-activated' cells were incubated with 100 μg/ml of FITC-LPA for 30 min at room temperature. After washing with MEM(−), the cells were fractionated on a FACS II (Becton-Dickinson Electronics Laboratory, Mountain View, CA).

Fractionation of 'LcA-activated' cells with LPA and PNA

10^8 'LcA-activated' cells in 0·5 ml of RPMI-1640 were incubated with 500 μg of LPA or PNA for 10 min at room temperature. The 'LcA-activated' cells were then fractionated by the method of Umiel, Linker-Israeli, Itzchaki, Trainin, Reisner & Sharon (1978). Briefly, the cells were layered over 50% inactivated foetal calf serum and after standing at room temperature for 60 min divided into three fractions; 'top', 'middle' and 'bottom'. The LPA or PNA which had bound to the fractionated cells was then washed away with 0·01 M D-glucuronic acid and 0·1 M lactose, respectively.

Treatment of LPA fractionated cells with anti-Thy-1·2 antiserum

Anti-Thy-1·2 antiserum was purchased from Litton Bionetico, Inc. (Kensington, MD). LPA-fractionated cells (10^7 /ml) which had been activated with LcA were

incubated with anti-Thy-1·2 (final dilution, 1:5) for 30 min at 4° followed by treatment with fresh rabbit serum (final dilution, 1:15) for 30 min at 37°.

RESULTS

Effect of the cells activated with various kinds of lectins on the anti-SRBC PFC response *in vitro*

The results in Fig. 1 show that the mitogens, such as Con A, PHA, PsA and WfM preferentially induce

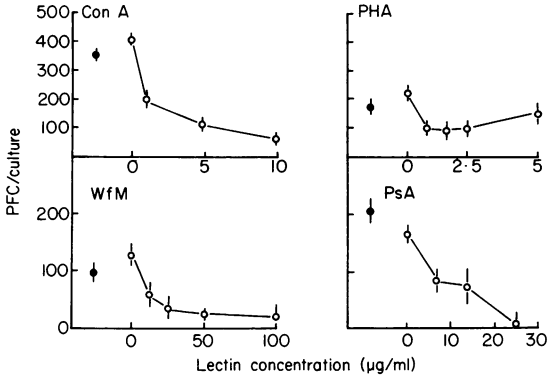

Figure 1. *In vitro* induction of suppressor cell activities with various kinds of lectins. Spleen cells (10^7 cells/ml) were cultured for 40 h with various kinds of lectins. The viable lymphocytes were purified and resuspended in RPMI-1640 medium. 2×10^5 of lectin activated cells were added to 2×10^6 fresh spleen cells plus 10^6 SRBC and cultured for 5 days. (•) Without addition of lectin-activated cells. Vertical bars represent SEM of triplicate experiments.

suppressor cell activity, rather than helper cell activity, under the experimental conditions. On the other hand, LcA or BpA were found to selectively induce helper activity. Various doses of LcA were incubated with mouse spleen cells for 40 h, and 'LcA-activated' cells ($2–8 \times 10^5$ cells) were co-cultured with normal fresh spleen cells (2×10^6 cells) plus SRBC (1×10^6 cells), primary anti-SRBC PFC being determined after incubation for 5 days. The results shown in Fig. 2 show that the 'LcA-activated' cells exhibited the strongest helper activity when the concentration of LcA used for the activation of the cells was 12·5 μg/ml. Both higher and lower concentrations of LcA than 12·5 μg/ml induced a smaller helper effect. The cells activated with the lectins such as PNA, LPA and WGA, however, had no effect on the primary antibody responses (Fig. 3).

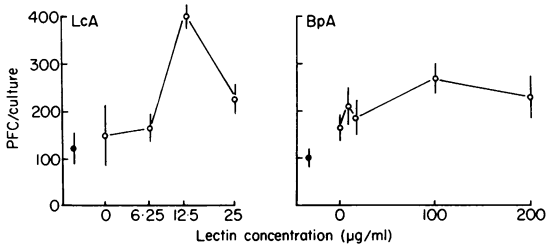

Figure 2. *In vitro* induction of helper cell activities with LcA or BpH. Cultures were performed as described in the legend to Fig. 1. (•) Without addition of lectin-activated cells. Vertical bars represent SEM of triplicate experiments.

Analysis of FITC-LPA labelled cells on a FACS II

'LcA-activated' spleen cells were labelled with FITC-LPA (100 μg/ml) and separated on a FACS II. Figure 4 shows a fluorescence profile, the cells with a relative fluorescence intensity of greater than 30 were defined as LPA positive (LPA^+) cells, and the remainder as LPA negative (LPA^-) cells. LPA^+ cells constituted 17% of the total 'LcA-activated' spleen cells under our experimental conditions. A size distribution profile (Fig. 5) showed that LPA^+ cells were composed of relatively small cells and that LPA^- cells mainly consisted of larger cells.

Functional analysis of the separated fractions

The cells separated on the FACS II were washed with RPMI-1640, and with 0·01 M D-glucuronic acid to remove LPA, since LPA is known to bind strongly to D-glucuronic acid (Nowak & Barondes, 1975). The washed cells were added to normal spleen cell cultures, and primary anti-SRBC direct PFC determined after cultivation for 5 days as described in Materials and Methods, unseparated 'LcA-activated' cells being added to the same culture system as a control. The cells which were stained with LPA had a marked helper effect, whereas the cells which were not stained with the lectin did not affect the antibody response (Table 1).

Separation of LcA-induced helper T cells by using LPA

'LcA-activated' cells were washed with 0·1 M α-methyl-D-mannoside. Then the washed cells were incubated with LPA for 10 min at room temperature. The cells were layered over 50% foetal calf serum. After 60 min at room temperature, the cells separated into three fractions, the 'top', 'middle' and 'bottom' fractions,

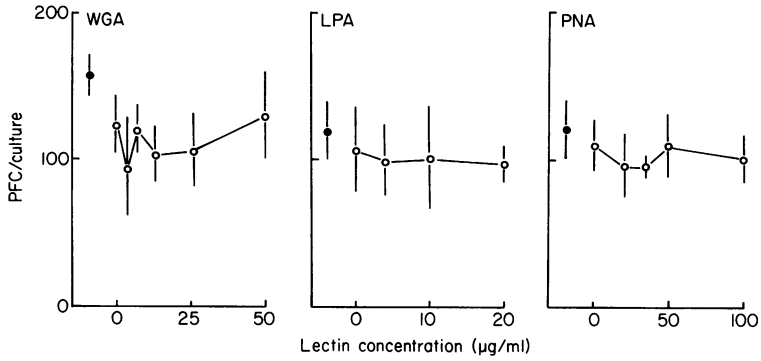

Figure 3. Effect of the cells activated with another group of lectins on immune responses. Spleen cells (10^7/ml) were cultured for 40 h with the lectins WGA, LPA and PNA. Then the cultures were carried out as described in the legend to Fig. 2. (●) Without addition of lectin-activated cells. Vertical bars represent SEM of triplicate experiments.

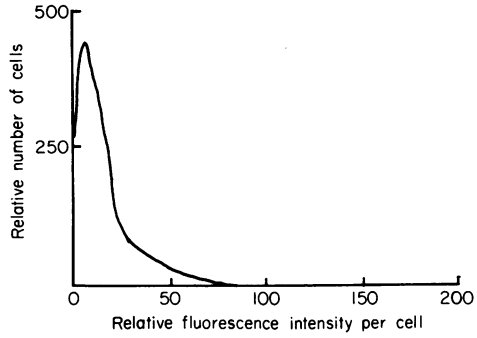

Figure 4. A fluorescence profile of 'LcA-activated' cells labelled with FITC-LPA. 'LcA-activated' cells were labelled with FITC-LPA and analysed on a FACS II as described in Materials and Methods.

respectively. The effect of these fractions on the primary antibody response to SRBC was tested. The LPA-agglutinating cells settled on the bottom of the tube exerted a significant helper effect, whereas the non-agglutinating cells which remained at the top of the tube exhibited no significant effect (Table 2).

When the LPA-agglutinating cells were treated with anti-Thy-1·2 serum and complement, the stimulatory activity of the cells was abolished (Table 2).

Separation of LcH-induced helper T cells by PNA

The 'LcA-activated' cells were incubated with PNA for 10 min at room temperature, and the PNA-treated cells were fractionated into three fractions as in the case of LPA-treated cells. Contrary to the results with LPA, non-agglutinating cells at the top of the tube exerted a strong enhancing activity to the primary antibody response to SRBC (Table 3).

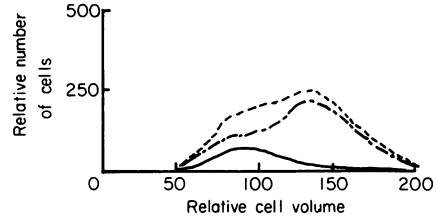

Figure 5. Size distribution profiles of 'LcA-activated' cells labelled with FITC-LPA. (– – –) Total population; (——) LPA^+ cells; (—·—·—) LPA^- cells. 'LcA-activated' cells were labelled with FITC-LPA and analysed on a FACS II as described in Materials and Methods.

Table 1. Effect of LcA-activated cells on primary PFC response to SRBC

Fraction	PFC/culture*	
	1×10^5†	4×10^5†
Control	92 ± 27	
Unseparated LcA-activated cells	144 ± 33	259 ± 131
LPA^- LcA-activated cells	97 ± 12	76 ± 20
LPA^+ LcA-activated cells	260 ± 78	373 ± 133

* PFC assays were carried out as described in the text. Each value represents the mean ± SEM of triplicate experiments.

† Number of LcA-activated cells added.

Table 2. Effect of LcA-activated cells on the primary PFC response to SRBC before and after fractionation with LPA*

Cells	Treatment	PFC/culture†
Control	—	152 ± 46
LcA-activated cells		
Before fractionation	—	200 ± 20
Top fraction	—	166 ± 16
Middle fraction	—	454 ± 46
Bottom fraction	—	502 ± 28
Bottom fraction	C′ alone	373 ± 44
Bottom fraction	Anti-Thy 1·2 + C′	162 ± 23

* Cell recovery in each fraction was as follows: top 10%, middle 71%, bottom 14%. The number of cells added to the culture from each fraction was 4×10^5

† PFC assays were carried out as described in the text. Each value represents the mean ± SEM of triplicate experiments.

Table 3. Effect of LcA-activated cells after fractionation with PNA*

Cells added	PFC/culture†
Control	99 ± 12
Top fraction	201 ± 20
Middle fraction	109 ± 13
Bottom fraction	42 ± 8

* Cell recovery in each fraction was as follows: top 13%, middle 68%, bottom 11%. The number cells added to the culture from each fraction was 4×10^5.

† PFC assays were carried out as described in the text. Each value represents the mean ± SEM of triplicate experiments.

DISCUSSION

The separation of suppressor and helper T cells has been performed by Jandinski, Cantor, Tadakuma, Peavy & Pierce (1976) and Okumura *et al.* (1977) using specific alloantisera, such as anti-Ly antiserum or anti-I-J antiserum. Tse & Dutton (1976) also reported that these two activities were separable on a Ficoll density gradient if the lymphocytes had been treated with Con A. The suppressor T cells were found to be fast sedimenting cells and the helper T cells were late sedimenting cells. On the other hand, Raff (1971) showed that T cells were composed of two functionally distinct cell populations at different maturation stages, i.e. T_1 and T_2 cells. T_1 cells are relatively short lived and immature. Furthermore, Gershon (1974) indicated that T_1 cells might correspond to the suppressor T cells, while T_2 cells might consist of helper T cells. Recently, Reisner *et al.* (1976) showed that a difference would be observed in the degree of substitution with sialic acids at the terminal positions of sugar chains on the cell surface, and that the immature cells corresponding to T_1 cells had a low sialic acid content compared to mature T cells. Since the suppressor T cells are supposed to be short lived (Lawrence & Schell, 1977), these reports support the theory that suppressor T cells form a part of the T_1-cell population.

In this study we have attempted to separate the helper T cells by using appropriate lectins. Recently, Erb, Meier, Kraus, von Boehmer & Feldmann (1978) showed that the helper cell precursors consist of a long lived T_2-cell pool, while the results of Reisner *et al.* (1976) showed that the cell surface of mature T cells, namely T_2 cells, is masked by sialic acid. Therefore, we investigated whether LPA which specifically binds to sialyl residues on the cell surface (Nowak & Barondes, 1975) could selectively discriminate against the T_2 cell pool of the helper T cells. We have also demonstrated that LcA, which has a subtle difference in sugar-binding specificity from that of Con A (Kaifu, Osawa & Jeanloz, 1975), could selectively activate the helper T-cell activity. Thus we used LcA as a non-specific inducer of helper T-cell activity, and attempted to fractionate 'LcA-activated' cells using LPA. In the size distribution profile (Fig. 5), LPA^+ cells which possessed the helper function were shown to be composed of smaller cells. This result is in good agreement with the work of Tse & Dutton (1976). We also demonstrated that the helper activity of LPA-agglutinating cells was sensitive to the treatment with anti-Thy-1·2 and complement (Table 2). These results indicate that the helper cells induced by the incubation with LcA are T cells and they have abundant sialic acid residues on the cell surface.

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