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## CHARACTERIZATION OF TWO MONOCLONAL ANTIBODIES WHICH RECOGNIZE DIFFERENT SUBPOPULATIONS OF CHICKEN T LYMPHOCYTES

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### SUMMARY

Distribution among peripheral T lymphocyte subpopulations and biochemical properties of the chicken lymphocyte surface antigens defined by monoclonal antibodies (mAbs) Lc-4 and Lc-6 were examined. Two-color immunofluorescence analysis revealed that Lc-4 and Lc-6 antigens were expressed on mutually exclusive subpopulations of peripheral T lymphocytes but not on B lymphocytes. Lc-4 mAb precipitated a polypeptide with apparent molecular mass of 35 and 65 kilodalton under reducing and non-reducing conditions, respectively. These results indicated that the antigen recognized with Lc-4 was closely similar in tissue distribution and biochemical property to mammalian CD8 antigen.

### INTRODUCTION

Chicken provides a unique model for the study of the development of lymphocytes because of the anatomical separation of primary lymphoid organs. But lack of the reliable surface markers of lymphocytes has limited the extensive studies of the development and function of chicken lymphocytes. Recently, a series of monoclonal antibodies (mAbs) have been established, which have identified the avian homologues of mammalian CD3 (3), CD4 and CD8 (2), and T cell receptors (5, 13).

We have been also established a panel of mAbs against chicken T lymphocyte surface antigens (7, 8). Among them, Lc-4 mAb reacted with 73% of thymocytes, 41% of spleen lymphocytes, and 23% of peripheral blood lymphocytes (PBL). Lc-6 mAb reacted with 75%, 17%, and 30% of cells from thymus, spleen, and PBL,

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respectively. Both mAbs had no reactivity against bursal lymphocytes. Lc-6 reacted with all T lymphoblastoid cell lines derived from Marek's disease tumors examined but Lc-4 did not.

In this paper, Lc-4 and Lc-6 mAbs were further characterized by two-color analysis on flow cytometry and immunoprecipitation. The possibility that Lc-4 and Lc-6 mAbs recognize the avian homologues of mammalian CD8 and CD4 antigens will be discussed.

#### MATERIALS AND METHODS

*Animals*: White Leghorn chickens were obtained from Aburahi Laboratories, Shionogi and Co. Ltd., Shiga, Japan. Japanese quails were obtained from a commercial source.

*Preparations of cells and immunofluorescence on flow cytometry*: Single cell suspensions were prepared as described previously (8). Immunofluorescence and flow cytometry (FCM) analysis was performed as described previously (7).

*mAbs*: Lc-4 and Lc-6 mAbs belonged to immunoglobulin (Ig) G1 and M, respectively, were used (7).

*Fluorochrome conjugation of Lc-6*: Lc-6 mAb was purified from ascitic fluid by ammonium sulfate precipitation followed by Sephacryl S-300 (Pharmacia-LKB, Uppsala, Sweden) gel filtration. Conjugation of Lc-6 with fluorescein isothiocyanate (FITC) was performed according to Goding (6).

*Two-color analysis on FCM*: Cells were first incubated with mAb for 30 min at 4°C. After washing with RPMI 1640 medium, cells were incubated with phycoerythrin (PE)-conjugated goat F(ab')<sub>2</sub> anti-mouse Ig G or M (Tago, Burlingame, CA) depending on the class of mAb, and then stained with FITC-conjugated goat anti-chicken Ig (Cappel, West Chester, PA) or FITC-conjugated Lc-6.

*Biochemical characterization of antigen recognized with mAb*: Radioiodination of thymocytes, immunoprecipitation, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were carried out as described previously (8).

#### RESULTS

*Distribution of the antigens recognized with mAbs among lymphocyte subpopulations*: Examination of reactivities of mAbs with cells from chickens of various ages showed that the frequency of Lc-4 and Lc-6 positive cells changed during the growth of chickens (Fig. 1). In PBL, the frequency of Lc-4 positive cells remained relatively constant during the observation period (9 to 131 days of age), while Lc-6 positive cells decreased from 50% (on day 10) to 20% (on day 100) (Fig. 1a). In spleen, the frequency of Lc-6 positive cells decreased as a function of age, while the frequency of Lc-4 positive cells progressively increased to reach about 60% in young adult chickens (Fig. 1b). These results suggest that the cells reactive with Lc-4 and Lc-6 belong to

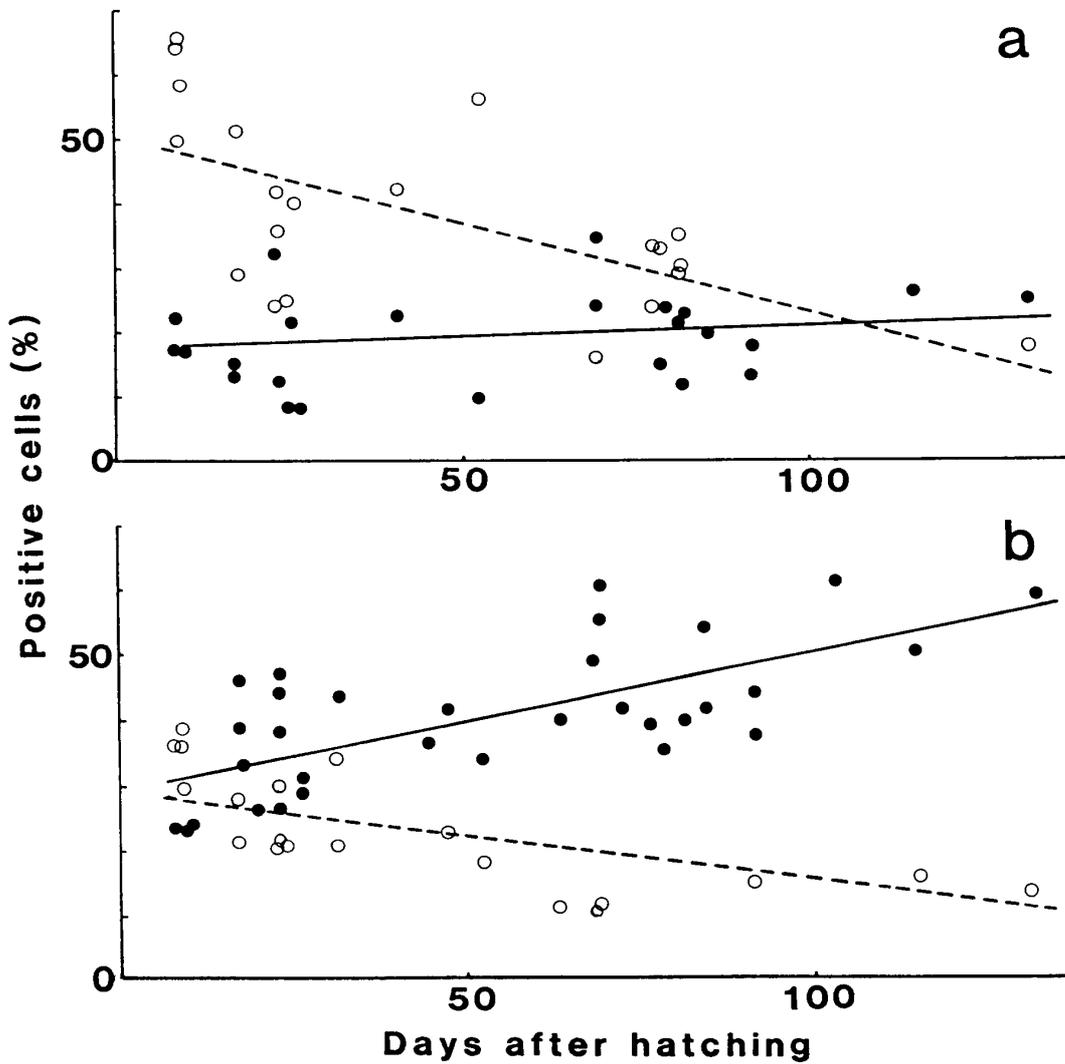


Fig. 1. Frequency of the cells from PBL (a) or spleen (b) reacted with Lc-4 (●, —) or Lc-6 (○, ·····). (a) Lc-4, n=24; Lc-6, n=20, (b) Lc-4, n=32; Lc-6, n=20.

different subpopulations. To confirm this, we examined the relationship between Lc-4 and Lc-6 positive cells by two-color analysis. The antigens recognized with Lc-4 and Lc-6 were expressed on discrete subpopulations of peripheral lymphocytes (Fig. 2a). Neither Lc-4 nor Lc-6 antigens were substantially expressed on surface Ig-bearing B lymphocytes (Fig. 2b & 2c).

*Biochemical characterization of Lc-4 and Lc-6 antigens:* Immunoprecipitation and SDS-PAGE analysis revealed that Lc-4 antigen was polypeptide with an apparent

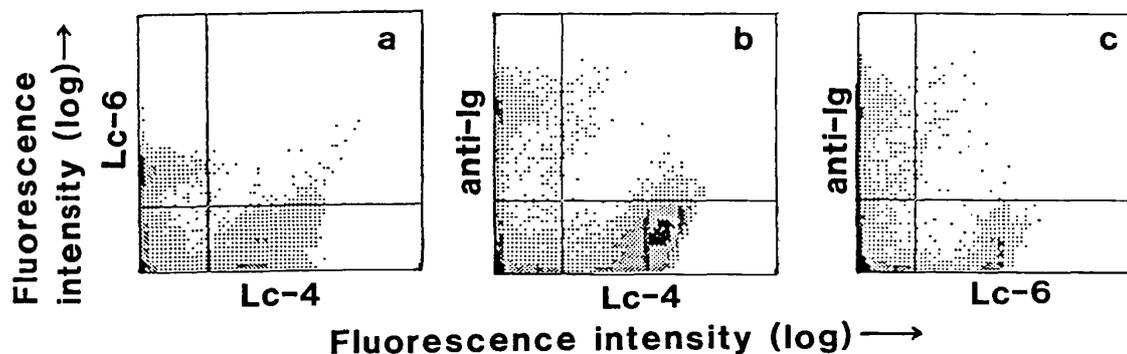


Fig. 2. Two-color analysis of the cells from PBL (a) or spleen (b and c). Combinations of mAbs and conjugates were as follows: (a) Lc-4, PE-conjugated anti-mouse IgG, and FITC-conjugated Lc-6, (b) Lc-4, PE-conjugated anti-mouse IgG, and FITC-conjugated anti-chicken Ig, (c) Lc-6, PE-conjugated anti-mouse IgM, and FITC-conjugated anti-chicken Ig.

molecular mass of 65 and 35 kilodalton (kDa) under non-reducing and reducing conditions, respectively (Fig. 3). Lc-6 mAb failed to precipitate any radiolabeled materials (data not shown).

*Reactivity of mAbs with quail thymocytes*: It has been shown that chicken lymphocytes expressed antigens which possess the antigenic determinants shared with chicken and quail (4, 10). We next examined the reactivity of Lc-4 and Lc-6 mAbs against quail thymocytes. As shown in Figure 4, Lc-4 reacted with quail thymocytes but Lc-6 did not.

#### DISCUSSION

We previously reported that Lc-4 and Lc-6 mAbs reacted with more than 70% of thymocytes and a portion of cells in spleen and PBL (7). In this paper, two-color analysis showed that Lc-4 and Lc-6 antigens were expressed on different subpopulations of peripheral T lymphocytes. In mammals, peripheral T lymphocytes can be divided into two subpopulations based on the expression of CD4 or CD8 antigen on their surface (11).

Lc-4 mAb precipitated a polypeptide of 35 and 65 kDa under reducing and non-reducing conditions, respectively, from radioiodinated thymocyte lysate. This indicates that Lc-4 antigen is composed of a disulfide-linked homodimer of 35 kDa polypeptide. The possibility, however, remained that Lc-4 antigen is a heterodimer of 35 kDa polypeptide and another subunit which could not be identified by the method in this study as indicated in mammalian CD8 antigen. CD8 antigen shows both

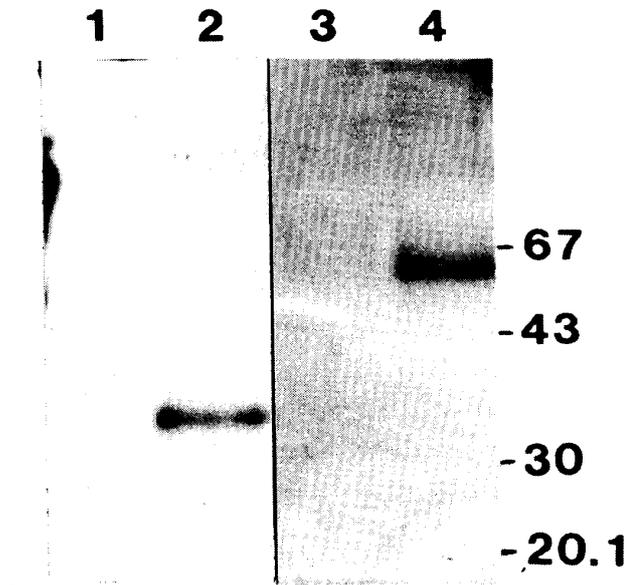


Fig. 3. SDS-PAGE analysis of the Lc-4 antigen. Radioiodinated thymocyte lysates were immunoprecipitated by M21 mAb (lane 1 and 3) or Lc-4 mAb (lane 2 and 4) under reducing (lane 1 and 2) or non-reducing (lane 3 and 4) conditions. M21 mAb, which recognizes Marek's disease virus antigen, was used as negative control. Molecular weights of marker proteins (kDa) are indicated on the right side of gels.

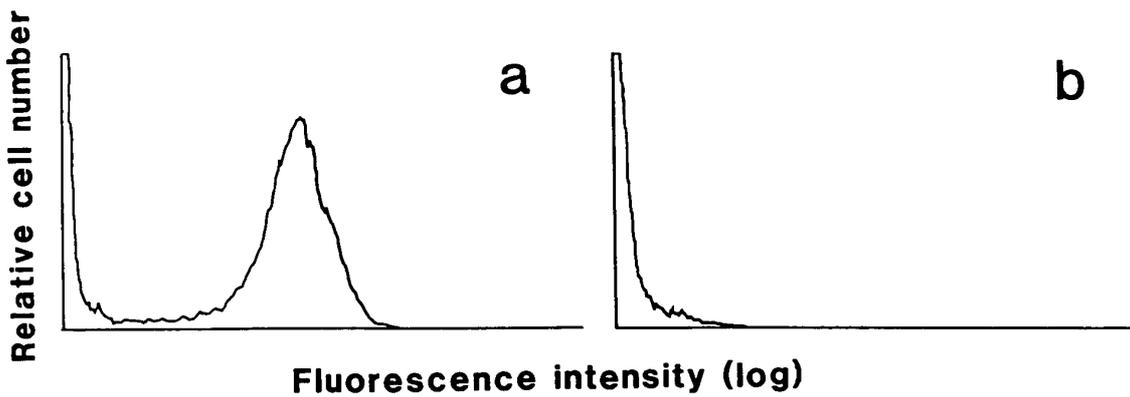


Fig. 4. FCM profiles of quail thymocytes stained with Lc-4 (a) or Lc-6 (b).

homodimeric and heterodimeric form composed of 30–38 kDa subunits (9, 12). Lc-6 antigen could not be identified in this study, but the antigen also seemed to be a polypeptide antigen because proteolytic enzyme treatment of cells could completely eliminate the reactivity of Lc-6 mAb (data not shown).

Recently, Chan et al. (2) also identified avian homologues of mammalian CD4 and CD8 antigens termed CT4 and CT8 antigens, respectively. CT8 antigen was a 63 kDa polypeptide composed of 34 kDa subunits and CT4 antigen was a 64 kDa single polypeptide. CT4 and CT8 antigens were expressed on more than 70% of thymocytes and on different subpopulations of peripheral T lymphocytes. The frequencies of the cells reactive with CT4 and CT8 mAbs were closely similar to those of the cells reactive with Lc-6 and Lc-4 mAbs, respectively (7). Comparison of our results with those of Chan et al. (2) strongly indicated that Lc-4 antigen was closely similar to CT8 antigen based on their tissue distribution and biochemical property. Although CT8 mAb did not react with quail lymphocytes (1), we found that Lc-4 reacted with quail thymocytes (Fig. 4), suggesting that Lc-4 and CT8 mAbs recognize the different determinants on the same antigen. On the other hand, tissue distribution study (7) and two-color analysis suggested that Lc-6 and CT4 mAbs recognize the same antigen, although we could not identify the antigen recognized with Lc-6 mAb. These results suggest that Lc-4 and Lc-6 mAbs recognize avian homologues of mammalian CD8 and CD4 antigens, respectively.

Lc-4 and Lc-6 mAbs should be useful in further studies of the chicken T lymphocyte development.

#### ACKNOWLEDGMENT

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