The Effects of Bovine T Lymphocytes Bearing Immunoglobulin G Receptors on the Plaque Forming Cell Response

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ABSTRACT. T lymphocytes bearing receptors for IgG (Tγ cells) in the human have been demonstrated to suppress immunoglobulin (Ig) synthesis by pokeweed mitogen (PWM)-stimulated normal B lymphocytes [8]. Human Tγ cells in the peripheral blood as well as in the lymphoid organs have been enumerated for the immunological analysis of some diseases including systemic lupus erythematosus and lymphoma [2, 4, 7]. Tγ cells in the bovine species have also been confirmed to be detectable in the peripheral blood [12]. However, the function of bovine Tγ cells has remained to be elucidated. The present paper deals with the effects of bovine Tγ cells from the peripheral blood on the development of plaque forming cells (PFC).

Heparinized peripheral blood was collected from a healthy female Holstein-Friesian cattle aged 6 years. Peripheral blood lymphocytes (PBL) were isolated by the Ficoll-Hypaque (Lymphoprep; specific gravity, 1.077, Daiichi-kagakuyakuhin, Japan) density gradient centrifugation method [10]. Initially, T lymphocyte-enriched fractions were separated from PBL by the method of Paul et al. [11]. The isolated PBL (5 x 10⁶ cells/ml) were mixed with sheep erythrocytes (E) (2.5 x 10⁸ cells/ml) treated with 2-aminoethyl isothiouronium bromide hydrobromide (AET; Sigma, USA). The mixed cells were incubated at 37°C for 15 min, centrifuged at 1000 rpm for 5 min, and then stood at 4°C for 12 hr. The proportion of E-rosette forming cells in PBL was 34.0%, when tested for E-rosette forming cells binding more than three AET-treated E in the mixed cells gently suspended. Subsequently, the suspended cells were separated into the two fractions of E-rosette forming cells and non-E-rosette forming cells by the density gradient centrifugation method using Lymphoprep. These two separated fractions were treated with a hypotonic tris-NH₄Cl (0.83%) solution. In PBL, E-rosette forming cell fractions, and non-E-rosette forming cell fractions, the proportion of B lymphocytes, monocytes and granulocytes was examined by the direct immunofluorescence method [9], acid alpha-naphthyl acetate esterase staining [6],
and Giemsa-staining, respectively. The proportion of B lymphocytes, monocytes, and granulocytes in PBL was 21.3%, 7.2%, and 1.5%, respectively, and that in the E-rosette forming cell fraction was less than 2% in all. As the result, the E-rosette forming cell fraction was regarded to be a T lymphocyte-enriched population. On the other hand, the non-E-rosett forming cell fraction consisted of 40.8% of B lymphocytes and less than 2% of monocytes and granulocytes, respectively, was regarded as a B lymphocyte-enriched population.

From the T lymphocyte-enriched population, Tγ cells and Tγ depleted cells (Tnon-γ cells) were separated by the erythrocyte-antibody (EA)-rosette sedimentation method [12]. The resultant T lymphocytes (2×10⁶ cells/ml) were mixed with an equal volume of bovine red blood cells (BRBC) (1×10⁸ cells/ml) coated with rabbit anti-BRBC serum IgG fraction (Cappel Lab., USA) diluted 1:64 with Eagle's minimum medium (MEM). The mixture was centrifuged at 1000 rpm for 5 min at 4°C and allowed to stand at 4°C for 60 min. After the incubation, an aliquot of the cells resuspended by gentle shaking, was layered onto a half volume of Lymphoprep, and then centrifuged at 1500 rpm for 40 min at 4°C. EA-rosette forming cells, sedimented at the bottom of the tube, were separated as Tγ cells, and non-EA-rosette forming cells, harvested from the interface of Lymphoprep, were as Tnon-γ cells. After treated with a hypotonic solution, Tγ and Tnon-γ cells suspended in RPMI-1640 (Gibco, USA) supplemented with 10% heat inactivated fetal bovine serum (Gibco, USA) and 20 μg/ml of gentamicin. Our preliminary experiment showed that the EA-rosette formation was inhibited by the pretreatment of T lymphocytes with IgG fraction of rabbit anti-BRBC serum (88 μg/ml), but not with IgM fraction (625 μg/ml).

B lymphocytes have been shown to differentiate to Ig-producing cells, after interacted with PWM and helper T lymphocytes [5]. To examine the helper function of T lymphocyte subsets, non-E-rosette forming cells (50×10³ cells) were mixed with 5—100×10³ of Tγ cells or Tnon-γ cells (as controls) in a final volume of 0.2 ml per well of a 96-well flat bottomed microtiter plate (Falcon, USA) containing 20 μl of PWM (50 μg/ml) (Sigma, USA). The plate was incubated for 5 days in a humidified atmosphere of 5% CO₂ in air. The number of PFC was determined by the reverse plaque assay as described by Gronowicz et al. [1] with a slight modification. Initially, one part of protein A (0.5 mg/ml) (Sigma, USA) was mixed with one part of packed sheep red blood cells (SRBC) washed with MEM and 10 parts of CrCl₃ (2.5×10⁻⁴ M). The mixture was incubated for 1 hr at 30°C and then centrifuged at 1500 rpm for 5 min at room temperature. The pellet SRBC were washed three times with 0.9% NaCl, and adjusted to a 25% suspension in MEM (protein A-coupled SRBC). Then, the mixture of 20 μl of the protein A-coupled SRBC solution, 100 μl of lymphocytes harvested from each culture well, 25 μl of rabbit anti-bovine IgG serum (Cappel, USA) diluted 1:20 with MEM, and 25 μl of fresh rabbit serum (as a source of complements) diluted 1:4 with MEM was prepared in a tube. Subsequently, 20 μl of the mixture were dropped on a slide glass and covered with a piece of cover glass as previously described by Hirono et al. [3]. After the slide glass was incubated for 5 hr at 37°C in a humidified atmosphere of 5% CO₂ in air, the hemolytic plaque appeared around Ig-producing cells. The number of the plaques was counted with a microscope (at a magnification of ×100). The coculture of non-E-rosette forming cells with Tnon-γ cells (100×10³ cells) resulted in a significant increase in the number of PFC as compared with that in non-E-rosette forming cells (NERFC) alone.
The enhancing effect of bovine T lymphocyte subsets on the development of PFC detected by the reverse hemolytic plaque assay. Each point and vertical bar is the mean and standard deviation of the number of PFC in triplicate cultures. (p<0.01) (Fig. 1). In contrast, the number of PFC decreased, when non-E-rosette forming cells were cocultured with Tγ cells (10–100×10^3 cells). This result shows that Tnon-γ cells are capable of providing an enhancing effect on the PFC response, but Tγ cells do not have such an effect.

For the further examination of the suppressive function of T lymphocyte subsets, Tγ cells and Tnon-γ cells were evaluated for the ability to inhibit the PFC response. The cell mixture of PBL (50×10^3 cells) and 5–100×10^3 cells of Tγ or Tnon-γ cells (as controls) was made in a final volume of 0.2 ml per each well of a 96-well flat bottomed microtiter plate containing 20 μl of PWM (50 μg/ml). After the plate was incubated for 5 days in a humidified atmosphere of 5% CO₂ in air, the number of PFC in cells harvested from each well was evaluated by the reverse hemolytic plaque assay. The addition of Tγ cells (50–100×10^3 cells) to PBL cultures resulted in the decreased number of PFC (p<0.05), while the number of PFC in PBL cocultured with Tnon-γ cells (100×10^3 cells) significantly increased as compared with that in PBL alone (p<0.01) (Fig. 2), suggesting that the Tnon-γ cell fraction contains a population of cells with helper function.

Therefore, these results indicate that bovine Tγ cells have suppressive effects on the PFC response in vitro, which may be due to the predominant presence of lymphocytes with suppressive function in the Tγ cell fraction. Considering the suppressive effect of bovine Tγ cells, the enumeration of Tγ cells will be expected to be one of useful means for the immunological analysis in cattle.
REFERENCES


要約

IgGレセプター保有ウシTリンパ球がブラーク形成細胞の反応に及ぼす効果（短報）：猪熊 純・廣田好和・長谷川篤彦・友田 勇（東京大学農学部家畜内科学教室）—Eロゼット沈降法およびEAロゼット沈降法によりウシの末梢血からIgGレセプター保有Tリンパ球（Ty）を分離し、TyとEロゼット非形成細胞または末梢血リンパ球を、PWM存在下37℃で5日間培養し、ブラーク形成細胞（PFC）をreverse hemolytic plaque assayにより検出した。Tyとの混合培養においてはPFC数の減少がみられ、TyはPFC発現に対して抑制効果を示すものと考えられた。