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Wild Type Rabies Virus Glycoprotein to Make Monoclonal Antibody for Early Detection with DAS-ELISA

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Abstract

The objective of this study was to search for wild type rabies virus glycoprotein G protein) for the production of monoclonal antibody for early detection with double antibody sandwich (DAS)-ELISA. This study was divided into several stages, including isolation and determination of type rabies virus with PCR, protein analysis with SDS-PAGE, immunogenicity and antigenicity tests, cloning with limiting dilution technique , purification of immunoglobulin, monoclonal antibody labeling, sensitivity and specificity test of monoclonal antibody used saliva and brain of mice with rabies virus infection. The result showed that the molecular weight of the G protein was 62–67 kDa. The result of cloning and recloning rabies virus showed that the virus was wild type (clone Jra 1– ra 8). Sensitivity values of monoclonal antibody used with DAS-ELISA from mice's brain was 95.23% and from mice's saliva was 97.62%. The specificity value of monoclonal antibody from mice's brain was 100% and from mice's saliva was 91.67%.

Keywords: rabies virus, G protein, monoclonal antibody and DAS-ELISA.

Introduction

Rabies is caused by a neurotropic virus belonging to genus *Lyssavirus*, family *Rhabdoviridae*, and is transmitted to all mammals. In Indonesia rabies is endemically distributed on the island of Sulawesi, Nusa Tenggara, Kalimantan and Sumatra. Vaccination and elimination program had been done, but the problem still uncovered. (Dibia, 2000; Sugiarto, 2001; Supriyadi *et al.* 2005)

Diagnosis to rabies cases, mainly in carnivora has many done by detection of antigen in brain samples (hippocampus, medulla oblongata, cortex cerebri or cerebellum) infected by rabies by the microscopic detection of negris bodies or via biologic test. This diagnosis by antigen detections is not practiced.

Antigen virus diagnosis can done with serologic tests, including indirect-FAT, virus neutralization, indirect ELISA, fluorescent antibodies virus neutralization (FAVN). (Kelly and Strik, 2000; Jackson *et al.*, 2000). In the field, these diagnostic methods are difficult to conduct because the wide geographic area of Indonesia (the islands of Sulawesi, Nusa Tenggara, Kalimantan and Sumatera).

This study attempted to produce a standard antigen diagnostic method of animal infected rabies using DAS–ELISA on saliva. DAS–ELISA used for antigen diagnosis is dependent on on sensitivity and specificity of monoclonal antibodies.

The objective of this study was to obtain wild type rabies virus glycoprotein for the production of monoclonal antibody to be used in early detection of the disease. The technique to be used is DAS-ELISA.

Methods

This study was divided on several stages, that is biological characterization of rabies virus in BHK – 21 cell lines, identification of virus by indirect ELISA and direct FAT, gene protein characterization by SDS – PAGE, and dot-blot technique. The Immunogenicity of protein coding was applied in mice. Molecular characterization by RT–PCR assays and analysis of PCR products was performed. Cloning and recloning monoclonal antibodies to G-protein wild type of rabies virus (Sambrook *et al.*, 1989; Suwarno *et al.*, 2002; Suwarno 2005).

Results and Discussion

Immunogenicity of G-protein is shown in Table 1. G-protein can induce antibodies titer reaching 10,240, while with whole molecule rabies virus the titre only reached 1,280. The high antibody titer with G-protein showed that it can be used to produce monoclonal antibodies.

Table 1: Immunogenecity of G-protein rabies virus to induce antibodies of mice by Indirect-ELISA

Kind of Antigen	Antibody Titre
G-protein	5120-10240
Whole molecule rabies virus	640-1280

Immunoglobulin subclass from monoclonal antibodies production indicated the success of cloning process. Immunoglobulin subclass from cloning and recloning is shown in Table 2.

Table 2: Immunoglobuline subclass to G-protein of rabies virus field isolates from cloning and recloning

Plate	Subclass Immunoglobulin		Clones name
	Cloning	Recloning	
1B2	Ig G1, Ig G2a	Ig G1	Jra-1
		Ig G2a	Jra-2
2D11	Ig G2b	Ig G2b	Jra-3
		Ig G2b	Jra-4
3H7	Ig M, Ig G3	Ig M	Jra-5
		Ig M	Jra-6
4G9	Ig G1, Ig G3	Ig G1	Jra-7
		Ig G3	Jra-8

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