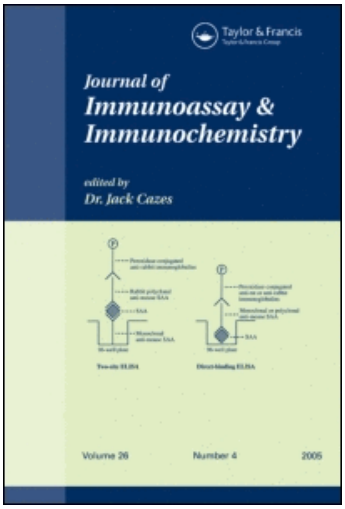

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Stability of Varicella-Zoster Virus and Herpes Simplex Virus IgG Monoclonal Antibodies

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Abstract: The stability of 3 monoclonal antibodies was analyzed at various temperatures and freeze/thaw cycles. Two varicella-zoster virus (VZV) IgGs (mAb 4F9 and mAb g62) and 1 herpes simplex virus 1 (HSV-1 mAb 1D4) were selected for these studies. IgGs were either incubated at various temperatures (25°C, 37°C, 45°C, and 60°C) for different periods of time (0 to 9 weeks) or processed for several freeze/thaw cycles. The reactivities of mAbs 4F9 (IgG1), g62 (IgG1) and 1D4 (IgG2b) were tested by indirect immunofluorescence assay (IFA). The results indicated that: (1) all three mAbs were stable at 25°C and 37°C for 9 weeks; (2) although the reactivities of mAbs g62 and 1D4 were diminished after 5 weeks, mAb 4F9 was stable at 45°C for 9 weeks; and (3) all 3 IgGs lost reactivity after overnight incubation at 60°C. In addition, the results showed that the reactivity of mAbs 4F9, g62 and 1D4 was not diminished after 12 freeze/thaw cycles.

Keywords: HSV, Monoclonal antibody, Stability, VZV

INTRODUCTION

The use of highly specific monoclonal antibodies is becoming more prevalent in the detection of viral infections including Varicella-zoster

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virus (VZV) and herpes simplex virus.[1,2] The nature of monoclonal antibodies results in varied sensitivity to numerous experimental conditions. These include sensitivity not only to assay changes in pH, buffer salts and ionic strength but also temperature and storage conditions.[3] Changes in these conditions may result in altered antibody-antigen affinity[4] which may adversely affect the assay with a decrease in sensitivity.[5] The goal of this study was to investigate the effects of temperature and storage conditions which may be encountered in storage, handling and the transportation of VZV and HSV monoclonal antibodies used for research and diagnostics.

EXPERIMENTAL

Monoclonal Antibody Production and Purification

Monoclonal antibodies (mAbs) to VZV glycoprotein gE (mAb 4F9) and immediate early protein 62 (mAb g62) were prepared as described previously.[6] Hybridoma cell line producing herpes simplex virus mAb 1D 4 [7] was obtained from ATCC (Item Number, HB-8068). Hybridoma cells were cultured *in vitro* in Iscove's Modified Dulbecco's Medium (Gibco) supplemented with 5.0% fetal bovine serum (FBS), antibiotic-antimycotic, glutamine, and gentamycin (Hyclone). The cell culture suspension was adapted into BD Cell® media (Becton Dickinson), which was supplemented with 10% FBS (Hyclone), according to manufacturer's protocol. Maximum antibody yield was obtained by transferring 500 mL of the cell suspension to a 490 cm^3 roller bottle, seeded at 2×10^6 cells per milliliter, and placed on a rolling apparatus for 2 weeks.

The cell culture fluid was centrifuged and filtered using Nalgene low protein binding filter units to remove any cellular debris and diluted 1:2 with 3 × loading buffer (1.5 M Na_2SO4, 300 mM glycine, 50 mM borate at pH 8.8). The samples were applied onto a 25 mL protein A column according to manufacturer's instructions and washed with five column volumes of loading buffer (Pharmacia). Immunoglobulin G (IgG) antibody was eluted with 100 mM glycine, pH 3.0 and neutralized with 1 M Tris-HCl, pH 8.0. The purified IgGs were analyzed using SDS-PAGE,[8] concentrated to 5 mg/ml in PBS using a 30 K filter (Amicon Centriplus), aliquoted and stored at −20°C.

Temperature and Freeze/Thaw Conditions

IgG samples were tested at a working concentration of 10 μg/mL. The samples were incubated at various temperatures (25°C, 37°C, 45°C,

60°C) overextended time periods (time 0–9 weeks) in a dry bath incubator (Fisher Scientific). The samples were tested using indirect immunofluorescence assay (IFA) as described.[6] For freeze/thaw experiments, aliquots (10 μg/mL) of each mAb were removed from storage (−80°C) and allowed to thaw to room temperature (24°C). The samples were tested using IFA and then returned to −80°C for additional freeze/thaw repetitions.

Indirect Immunofluorescence Assay (IFA)

Reactivities of mAbs were tested using VZV and HSV antibody control slides (Bion) each containing one well of uninfected cell and one VZV- or HSV-infected cell monolayer. Antibody samples (100 μL) were added to each well of the control slide, one containing uninfected cells, the other VZV- or HSV-infected cells. The slides were incubated at 37°C for 30 min in a CO_2 incubator. Slides were washed twice with cold 0.01 M phosphate buffer saline solution, pH7.4 (PBS) and then incubated with fluorescein-conjugated (FITC) goat anti-mouse IgG (1:30 dilution) at 37°C for 30 min. The slides were washed twice with PBS, rinsed with water, dried at room temperature, mounted using a 50% glycerin/PBS solution and observed under a fluorescent microscope (Leitz). The fluorescent intensity of each slide was graded using the Immunofluorescence Test on a 4+ scale.[9]

RESULTS

Effect of Temperatures on Stability of Antibodies

The effect of various temperatures on the stability of mAbs 4F9, g62, and 1D4 IgGs is shown in Table 1. The results indicated that these IgGs are stable for at least 9 weeks at 37°C. VZV mAb 4F9 IgG1 was also stable at 45°C for 9 weeks, however, the reactivity of VZV g62 IgG1 and HSV-1 mAb IgG2 (1D4) was reduced to 2+ and 3+, respectively, after 4 weeks at 45°C. All 3 mAbs lost their reactivity at 60°C after 1 week. Additional experiments have determined that at 60°C there was complete loss of reactivity between 24 and 48 hours.

Effect of Freeze/Thaw Cycles on Stability of Antibodies

Antibody samples were frozen at –80°C, thawed for five minutes in a 37°C water bath, tested, and re-frozen. As shown in Table 2, the antibodies

Table 1. Indirect immunofluorescence assay (IFA) test results for VZV and HSV IgGs at various temperatures

		Time (weeks)									
Antibodies	Temperature (°C)	0	1	2	3	4	5	6	7	8	9
4F9 IgG1	25	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	37	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	45	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	60	4+	0	0	0	0	0	0	0	0	0
Gene62 IgG1	25	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	37	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	45	4+	3+	3+	3+	2+	0	0	0	0	0
	60	4+	0	0	0	0	0	0	0	0	0
HSV-1 IgG2b	25	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	37	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	45	4+	3+	3+	3+	3+	3+	2+	2+	2+	2+
	60	4+	0	0	0	0	0	0	0	0	0

Grading Intensity: 4+, Glaring yellow-green fluorescence; 3+, Bright yellow-green fluorescence; 2+, Dull yellow-green fluorescence; 1+, Very dim yellow-green fluorescence; 0, No staining.

4F9 IgG1, gene 62 IgG1 and HSV-1 IgG2b were stable and did not lose their reactivity after 12 freeze/thaw cycles.

DISCUSSION

The extensive use of monoclonal antibodies (mAbs) in immunoassays has demonstrated many of their advantages, including antigen specificity, unlimited supply and ease of purification. These qualities have made mAbs excellent tools for research and diagnostic applications.[10,11] As is

Table 2. Indirect immunofluorescence assay (IFA) test results for VZV and HSV IgGs after different freeze/thaw cycles

	Freeze/thaw cycles											
Antibodies	1	2	3	4	5	6	7	8	9	10	11	12
4F9 IgG1	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Gene62 IgG1	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
HSV-1 IgG2b	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+

Note: Grading intensity has been described in Table 1.

characteristic of most proteins, monoclonal antibodies are sensitive to certain conditions which may adversely affect their activities. The physical stress of freeze/thaw cycles or excessive heating may denature the antibody molecule and can contribute to loss of reactivity.[12] The results presented in these preliminary studies showed that all three mAbs were stable; mAb 4F9 was most stable when exposed to various conditions including elevated temperatures and multiple freeze thaw cycles.

The experiments described here were designed to simulate possible storage conditions and determine their effect on the activity of these mAbs. While it is a common practice to avoid multiple freeze thaw cycles and unnecessary heating of antibody reagents, unintentional extreme conditions may occur. Unfortunately, preventative practices such as multiple aliquoting and redundant cooling systems may not always be practical or available for storage. Inconsistent shipping methods including dry or wet ice, cold packs or at ambient temperatures may also exacerbate the problem.

Purified monoclonal antibodies are widely used for biomedical research and diagnostic testing. The detection of glycoproteins on the surface of infected cells has been a tool for determining viral infection. MAbs 4F9 (IgG1), g62 (IgG1), and 1D4 (IgG2b), have both the ability to be used for determination of VZV and HSV exposure and the advantage of being very stable. These characteristics of specificity and physical stability make these mAbs a useful tool for in vitro diagnostic applications and post-immunization screening.

ABBREVIATIONS

mAb, monoclonal antibody; IFA, indirect immunofluorescence assay; VZV, varicella zoster virus; HSV, herpes simplex virus; FBS, fetal bovine serum.

DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

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