IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reexamination of Patent No. 6,331,415	)	
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Shmuel CABILLY et al.	)	Group Art Unit: 3991
	)	
Control No. 90/007,542	)	Examiner: Bennett M. Celsa
	)	
Filed: May 13, 2005	)	Confirmation No.: 7585
	)	
For: METHODS OF PRODUCING IMMUNOGLOBULINS, VECTORS AND TRANSFORMED HOST CELLS FOR USE THEREIN	)	

DECLARATION OF DR. DOUGLAS A. RICE UNDER 37 C.F.R. § 1.132

I, Douglas A. Rice, declare as follows:

1. I am a citizen and resident of the United States of America. A copy of my *curriculum vitae* is attached as Exhibit A to this declaration.

2. I am the same Douglas Rice listed as the first author on the paper entitled "Regulated expression of an immunoglobulin κ gene introduced into a mouse lymphoid cell line," published at *Proc. Nat'l Acad. Sci. USA* 79: 7962-65 (1982).

3. I have reviewed the Office Action mailed September 13, 2005, in the reexamination of U.S. Patent No. 6,331,415, and the references cited in that communication.

4. I have been retained by the owners of the '415 patent, City of Hope and Genentech, Inc., to comment on the Examiner's characterization of my 1982 *PNAS* paper and its relevance to the claims of the '415 patent.

5. At page 6 of the Office action, the Examiner states:

> Rice et al successfully introduced a recombinant rearranged murine kappa light chain gene construct into an Abelson murine leukemia virus (A-MuLV)- transformed lymphoid cell line, which is a cell line that already synthesized γ2b heavy chain protein (see page 7862). Rice inserted the light chain gene into a plasmid, used this plasmid to transfect the cells, and then examined the polypeptides as well as the RNA produced by the cells (see pages 7863-7864, and Figures 2 and 3). Lastly, since the cells were producing both immunoglobulin chains, the cells were examined for the ability to assemble the chains into IgG molecules, leading to the

> observation that "[e]ssentially all of the κ chain produced in the κ-2 cells appears to be assembled into lgG2b." (see page 7864). Thus, at the time of filing the application for the '567 [sic] and '415 patent it was known in the art that host cells could express "heavy or light chains," and that expression of both chains was routine, resulting in assembly into immunoglobulins.

6. For the following reasons, I do not agree with the Examiner's conclusions concerning my paper.

7. The focus of my research in David Baltimore's laboratory was immunoglobulin (Ig) gene regulation in lymphoid cells. Our objective was to develop an experimental platform for understanding the molecular mechanisms associated with the regulation of Ig gene expression. We were not attempting to develop a system for recombinantly producing immungolobulins.

8. In our 1982 *PNAS* paper, we reported that a rearranged kappa (κ) light chain gene could be introduced into a lymphoid cell line and expressed. As we stated in the paper (page 7864), "[t]his opens the possibility of examining control and rearrangement mechanisms [for Ig genes] in lymphoid cells by using inserted genetic elements."

9. We chose the 81A-2 cell line for use in our experiments because it exhibited a relatively mature B cell phenotype and expressed a heavy chain gene but not a light chain gene. As we explained in the introduction to our paper:

> B cell differentiation proceeds from the "pre-B" lymphocyte, which synthesizes μ immunoglobulin (Ig) heavy chains but no light chains, to the mature B lymphocyte, which synthesizes both heavy and light chains and expresses surface Ig, and finally to the Ig-secreting plasma cell. The availability of transformed cell analogs has allowed biochemical characterization of these stages of cellular differentiation.

10. The degree of maturation of the B cells in the 81A-2 cell line was an important factor in our decision to use this cell line. The 81A-2 line is a subclone of the 18-8 cell line. Because the parental 18-8 cell line is capable of expressing its endogenous κ light chain gene, we hoped that the 81A-2 subclone would be poised to express an exogenous functionally rearranged light chain gene. We thus designed our experiments to investigate whether a virus-transformed cellular analog of a lymphoid cell at the proper stage of development, exemplified in our work by the 81A-2 subclone, would be a good model system for studying the regulation of light chain gene expression.

11. The transformant we describe in the paper was not designed to produce a functional immunoglobulin that would bind antigen. The κ chain transformant described in the paper is only capable of producing the gamma-2b (γ2b) heavy chain protein encoded by 81A-2 genomic DNA and the exogenous κ light chain protein encoded by the murine MOPC41 κ light chain gene that we introduced into the cell. Because the antigen binding specificities of the heavy chain and light chain polypeptides were different, there was no

expectation and it is unlikely that expression products of this cell line would exhibit selective antigen binding activity.

12. My paper did not discuss expression of exogenous Ig heavy chain genes in lymphoid cells. In our experiments, we did not attempt to insert an exogenous heavy chain gene along with the exogenous light chain gene we inserted into the 81A-2 subclone. In fact, the 81A-2 host cell would have been an inappropriate host cell for this purpose because it already expressed an endogenous heavy chain.

13. It never occurred to us, as part of our work leading to the results published in the *PNAS* paper, to attempt to express exogenous heavy and light chain genes in the 81A-2 cell line.

14. The Examiner is incorrect, and I disagree with the suggestion, that by early April 1983, my *PNAS* paper had made routine or predictable the task of expressing exogenous immunoglobulin light and heavy chain genes in the same cell. In later experiments, I attempted to use the techniques described in the *PNAS* paper to introduce and express single Ig genes into other lymphoid cell lines. Most of these experiments failed to produce stable transfectants. Thus, my experience was that using the same transfection and selection conditions described in the *PNAS* paper with other cell lines or other Ig genes did not routinely yield stable transformants containing even a single exogenous Ig gene.

15. I also was not aware of any published reports by early April 1983 describing the introduction and expression of both immunoglobulin heavy and light chain genes into a single host cell. As of that date, I also was not aware of any groups attempting to introduce and express both immunoglobulin heavy and light chain genes into a single host cell.

16. Finally, my paper did not establish that the exogenous Ig light chain and the endogenous heavy chain polypeptides were properly assembled in the 81A-2 transformant into an Ig tetramer with antigen-binding activity. While the paper reports the presence of a higher molecular weight product that is approximately the size of an H_2L_2 tetramer, as shown in Figure 4 of the paper, we did not in any way prove these were antigen-binding H_2L_2 tetramers. On the contrary, we had no way to predict what the antigen-binding properties of such a product would be because, as discussed above, the antigen-binding specificities of the component heavy and light chain polypeptides were different.

17. In view of these observations, I do not agree with the Examiner's suggestion that my paper explains how to produce a lymphoid cell line that expresses exogenous heavy chain and light chain immunoglobulin genes. I did not demonstrate in my paper that one could transfect a host cell with both heavy and light chain genes. My paper also does not establish that the transfected cell line forms properly assembled immunoglobulin tetramers out of the endogenous heavy chain and exogenous light chain. My paper does not explain how one might make transfected host cells that produce immunoglobulins formed from the products of independently expressed exogenous heavy and light chain genes. In view of the inherent limitations of the experimental system described in my paper and the uncertainties we describe in our conclusions, I simply do not agree with the

Examiner that my paper made it routine to transfect lymphoid cell lines with exogenous immunoglobulin heavy and light chain genes.

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I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the patent subject to this reexamination proceeding.

Douglas A. Rice

Nov. 23, 2005
Date