

Tribute to Baruch Blumberg

Baruch Blumberg's spirit left Earth in the ideal way. In Hebrew we use the words *mot beshikar* – *death with a kiss and with no suffering*.

Baruch was a remarkable man. A scholar, an administrator, a leader and a visionary, and he was modest. He had a tremendous enthusiasm for exploring – he had his feet on the ground and his head in the stars. Both his public and private life related to the same person. The warmth and general affection that he showed to all his friends and colleagues and his kindness, sensitivity, together with his humour, made Baruch an exceptional man who was universally liked.

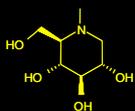
He combined intellectual and personal honesty, kindness, professional integrity, a great deal of common sense, imaginative skills, goodness of heart to everyone. He was widely respected throughout the world and many countries, such as Korea, which practically worshipped him, since his introduction of his vaccine for Hepatitis B has saved millions of lives. He had a sense of fun and mischief and of honour that made being with him so exciting and so exhilarating. He loved interacting with United Therapeutics. He loved the company.

We shall celebrate and remember Baruch's achievements. We shall mourn the loss of a great friend and scientist. He leaves, as it says in the Talmud, the greatest crown of all – the crown of a good name. To paraphrase Yates: our glory was, we had such a friend.

--Professor Raymond Dwek, FRS

Baruch Blumberg-- The Oxford Connection-- A Tribute

Mulberry Tree



iminosugar

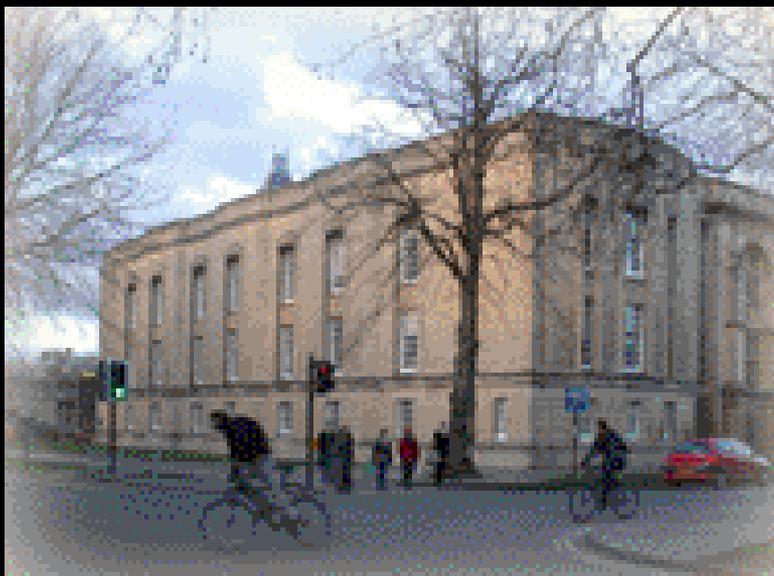


Master of Balliol 1989 - 1994

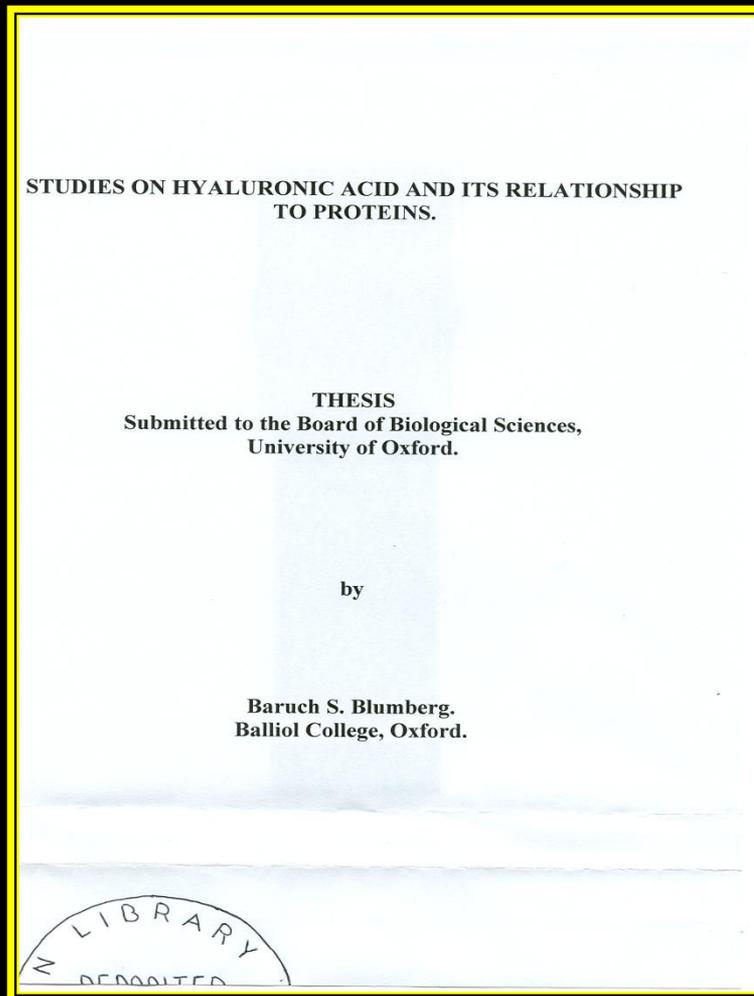
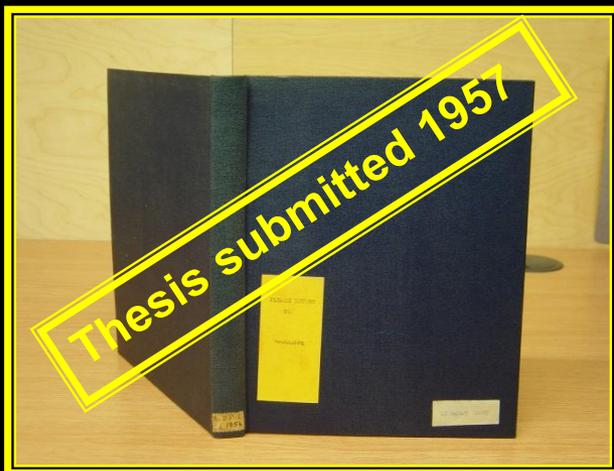


Professor Baruch Blumberg

- **Postgraduate 1955 – 1957**
- **Visiting Fellow of Trinity 1972**
- **Nobel Prize Winner 1976**



Radcliffe Science Library



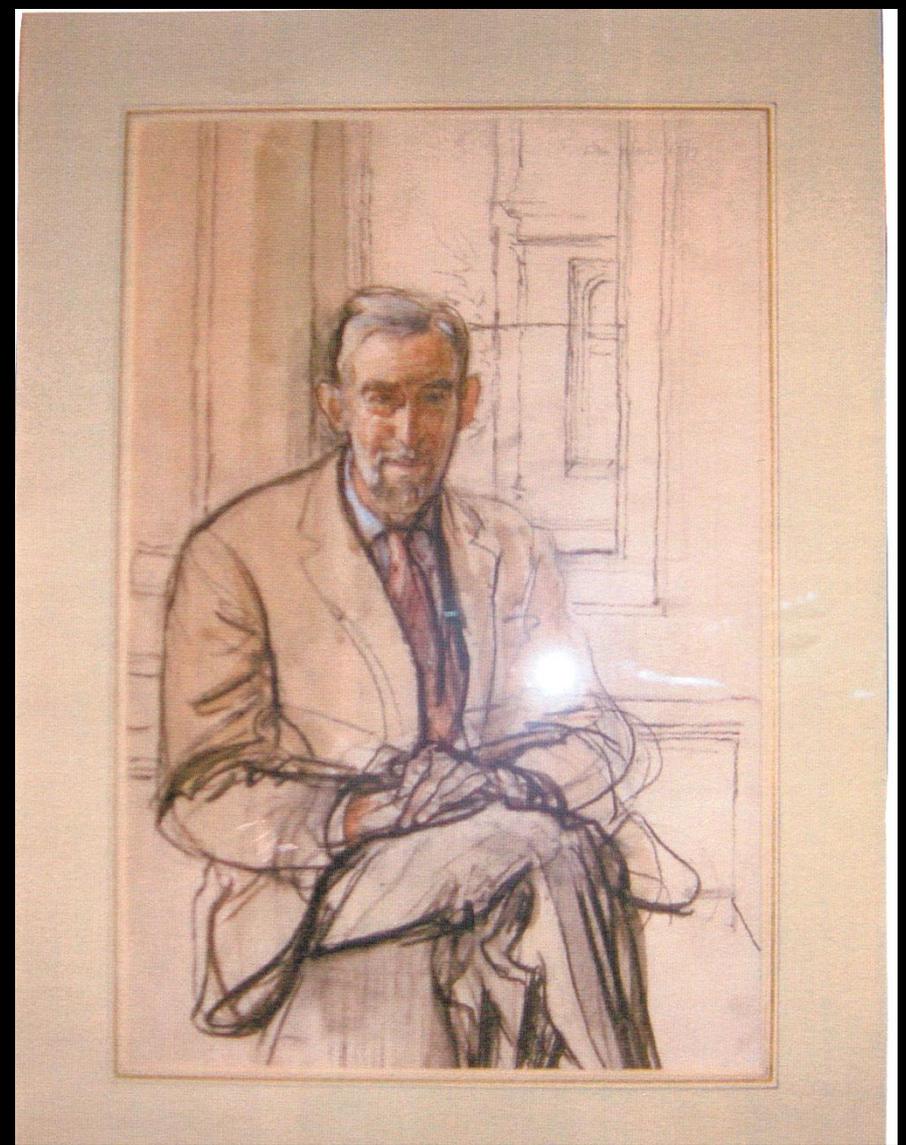
Professor Alexander Ogston

1911 - 1996



President of Trinity College

1970 - 1978



“his scientific bent was for sensible solutions”

“I was one whom he influenced.

His scientific style had an enormous effect on mine”.. Memorial Oration by BSB

Hepatitis B virus (HBV) envelope glycoproteins vary drastically in their sensitivity to glycan processing: Evidence that alteration of a single N-linked glycosylation site can regulate HBV secretion

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Contributed by Baruch S. Blumberg, December 17, 1996

ABSTRACT The role of N-linked glycosylation and glycan trimming in the function of glycoproteins remains a central question in biology. Hepatitis B virus specifies three glycoproteins (L, M, and S) that are derived from alternate translation of the same ORF. All three glycoproteins contain a common N-glycosylation site in the S domain while M possesses an additional N-glycosylation site at its amino terminus. In the presence of *N*-butyl-deoxojirimycin (an inhibitor of α -glucosidase) virions and the M protein are surprisingly retained. Preliminary evidence suggests that the retained M protein is hyperglycosylated and localized to lysosomal vesicles. In contrast, the S and L proteins are secreted, and their glycosylation state is unaffected by the presence of the inhibitor. Site-directed mutagenesis provides evidence that virion secretion requires the glycosylation sequon in the pre-S2 domain of M. This highlights the potential role of the M protein oligosaccharide as a therapeutic target.

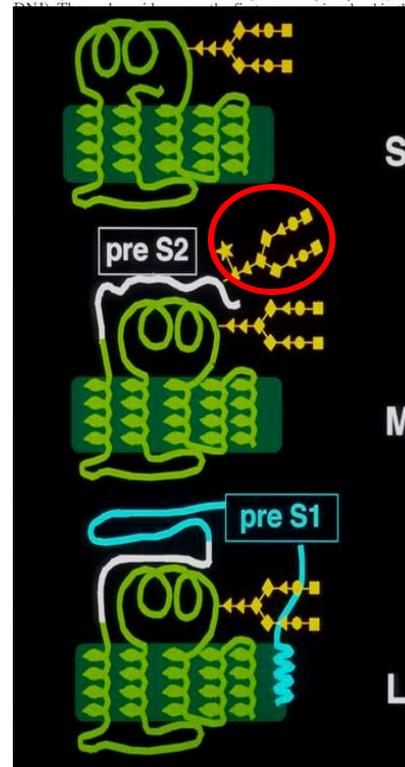
Hepatitis B virus (HBV) is the human member of the *hepadnaviridae* family of viruses which infects over 300 million people worldwide. The HBV genome encodes for three related envelope proteins termed L, M, and S (Fig. 1A). The three envelope proteins are produced from a single ORF through alternative translation start sites. All three proteins have a common N-linked glycosylation site at position 146 of the S domain (Fig. 1A), while the M protein alone contains an additional site at position 4 of the pre-S2 domain (1).

A peculiar feature of these envelope glycoproteins is that they are secreted in the form of small, noninfectious, non-DNA-containing, lipoprotein particles. These subviral particles are secreted in vast numbers, often outnumbering the infectious HBV virion by 100,000:1 (1). Subviral particles are found in two forms: spheres, which consists mainly of the S and M proteins; and filaments, which contain a greater amount of the L protein (2).

All three envelope glycoproteins are important in the viral life cycle. While it has been shown that the L and S proteins are necessary for virion secretion (3), the role of M is in doubt (3, 4). The current model of virion formation involves DNA containing nucleocapsids budding into the lumen of the endoplasmic reticulum (ER) and secretion through the trans-Golgi network (5).

The N-linked glycosylation pathway is well defined, consisting of over 13 enzymes that are involved in processing within the ER and the Golgi apparatus (6). Specific inhibitors of this pathway can be used to probe the importance of N-linked glycosylation. The role of N-linked glycosylation in HBV has

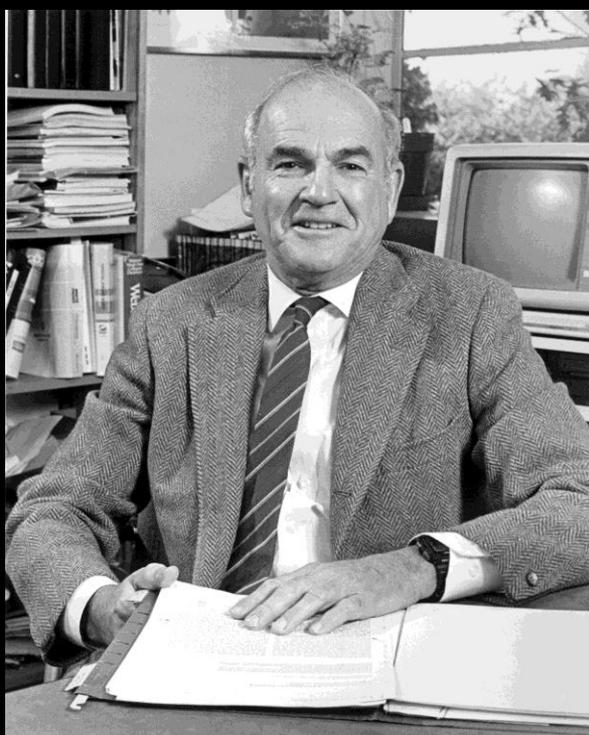
yet to be determined. Several reports have provided evidence that the N-linked glycans are not necessary for the secretion of the subviral particle (7, 8). In contrast, the secretion of virus requires both N-linked glycosylation (9) and N-linked glycan processing (10). However, it was unclear whether this resulted from a general effect of glycosylation or from a specific effect on a particular viral glycoprotein. To this end, we have now analyzed the glycosylation state and the secretion levels of the three envelope glycoproteins (S, M, and L) in the presence of the α -glucosidase inhibitor *N*-butyl-deoxojirimycin (NB-DNJ).



Abbreviations: HBV, hepatitis B virus; ER, endoplasmic reticulum; NB-DNJ, *N*-butyl-deoxojirimycin.

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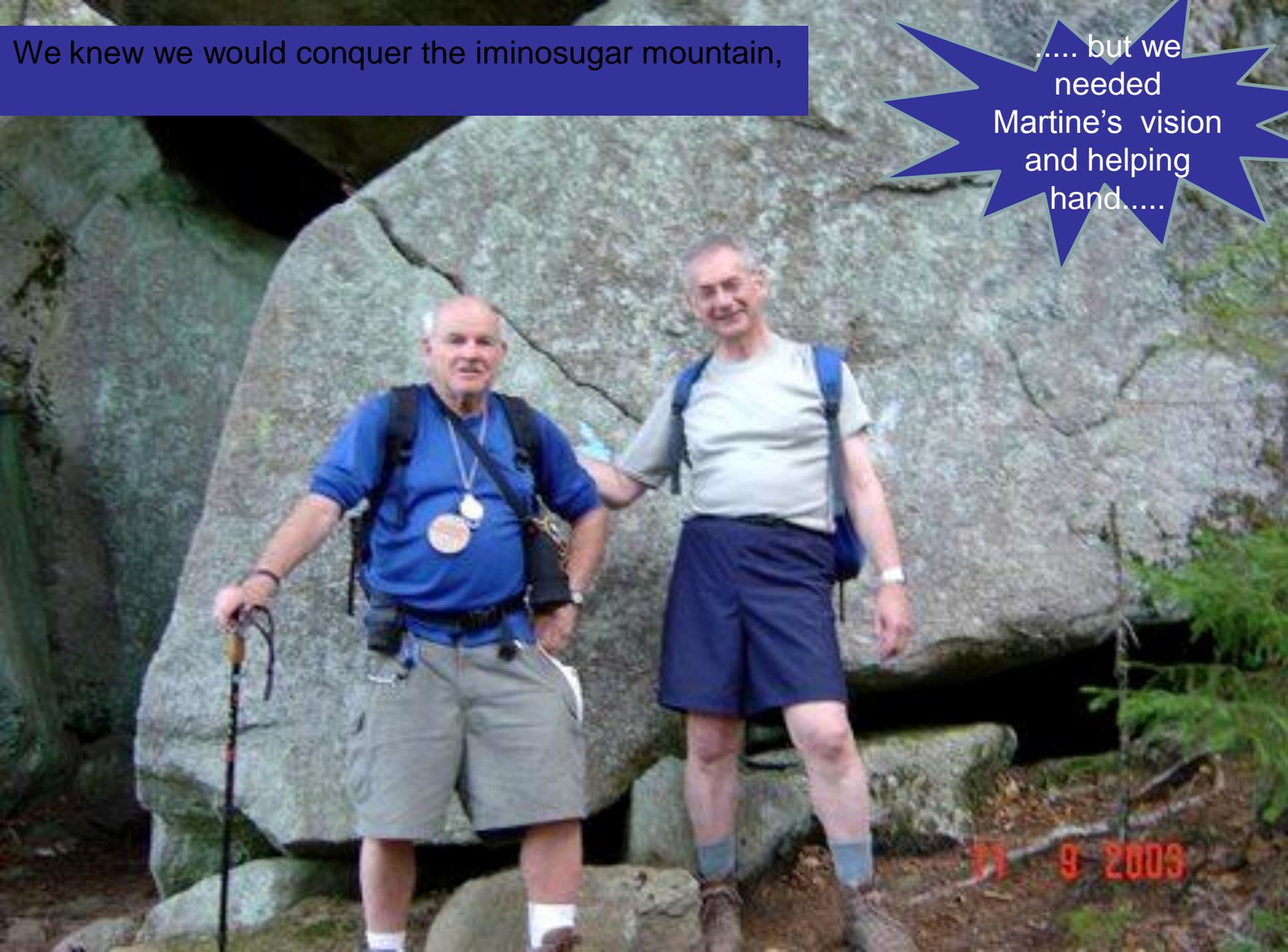


1989-BARUCH BLUMBERG JOINS THE GLYCOBIOLOGY INSTITUTE



We knew we would conquer the iminosugar mountain,

..... but we needed Martine's vision and helping hand.....



11 9 2003

2005



The Chancellor, Masters and Scholars of Oxford University send greetings to Baruch Blumberg on the occasion of his 80th birthday and acknowledge his achievements in medicine, scholarship, friendship and participation in Oxford University.

A handwritten signature in blue ink, appearing to read "C. Patten".

The Rt Hon the Lord Patten of Barnes, CH
Chancellor - University of Oxford

A handwritten signature in blue ink, appearing to read "Raymond Dwek".

Professor Raymond Dwek
Professor of Glycobiology



Geographic Distribution of HBV Infection

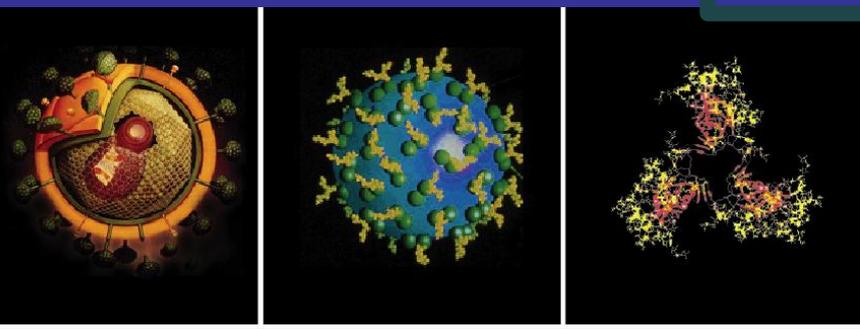


Estimated that 20M lives saved

One billion people vaccinated

- 2 billion people infected
- 350 million are carriers and at high risk of chronic liver disease & liver cancer

■ > 8% High
■ 2-7 % Intermediate
■ < 2 % Low



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SPEAKERS
Tim Cook and Tom Hookaday
Isis Innovation Ltd.
(the technology transfer company of the University of Oxford)

COMMENTS by
David Billington, Princeton University
Baruch S. Blumberg, American Philosophical Society
Mark S. Frankel, Ph.D., AAAS
and Raymond Dwek, FRS, Chair of Technology and Society
John W. Kluge Center at the Library of Congress

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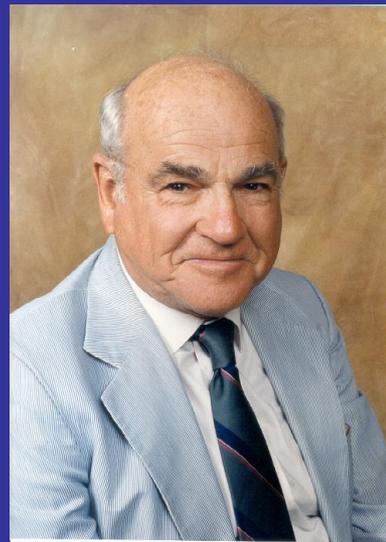


Opening Unither Antiviral Unit 2006

Oxford



First Unither Virology Lecture 2006

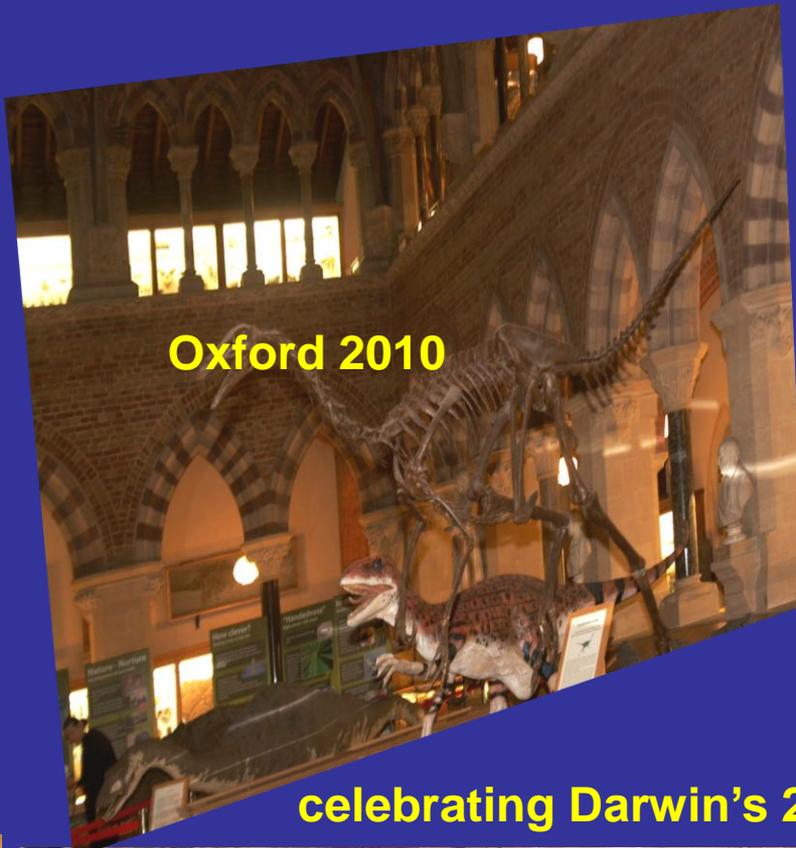


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celebrating Darwin's 200th birthday



Polyunsaturated liposomes are antiviral against hepatitis B and C viruses and HIV by decreasing cholesterol levels in infected cells

Stephanie Pollock^a, Norica Branza-Nichita^b, Annette Böhmer^a, Cristina Radulescu^b, Raymond A. Dwek^a, and Nicole Zitzmann^{a,1}

^aOxford Antiviral Drug Discovery Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom; and ^bInstitute of Biochemistry, Romanian Academy, Bucharest 060031, Romania

Communicated by Baruch S. Blumberg, Fox Chase Cancer Center, Philadelphia, PA, June 30, 2010 (received for review May 20, 2010)

The pressing need for broad-spectrum antivirals could be met by targeting host rather than viral processes. Cholesterol biosynthesis within the infected cell is one promising target for a large number of viral systems, including hepatitis C virus (HCV), hepatitis B virus (HBV) and HIV. Liposomes developed for intracellular, endoplasmic reticulum (ER)-targeted *in vivo* drug delivery have been modified to include polyunsaturated fatty acids that exert an independent antiviral activity through the reduction of cellular cholesterol. These polyunsaturated ER liposomes (PERLs) have greater activity than lovastatin (Mevacor, Altoprev), which is clinically approved for lowering cholesterol and preventing cardiovascular disease. Treatment of HCV, HBV, and HIV infections with PERLs significantly decreased viral secretion and infectivity, and pretreatment of naïve cells reduced the ability of both HCV and HIV to establish infections because of the decreased levels of plasma membrane cholesterol. Direct competition for cellular receptors was an added effect of PERLs against HCV infections. The greatest antiviral activity in all three systems was the inhibition of viral infectivity through the reduction of virus-associated cholesterol. Our study demonstrates that PERLs are a broadly effective antiviral therapy and should be developed further in combination with encapsulated drug mixtures for enhanced *in vivo* efficacy.

antivirals | endoplasmic reticulum targeting | liposome

A number of viruses depend on cholesterol to maintain a certain level of fitness. Thus, drugs that target this process should be useful in treating a broad variety of viral infections. Here we focus on three important human pathogens—hepatitis C virus (HCV), HIV, and hepatitis B virus (HBV)—which together exact a heavy toll on public health. All three viral infections require associations with cholesterol during at least one stage of their life cycle. Most of the viral life cycle of HCV is closely associated with lipid and cholesterol metabolism in host cells; this association includes entry into naïve cells (1), RNA replication (2), viral assembly (3), and infectivity (4). HIV relies heavily on lipid rafts for entry, assembly/secretion, and infectivity (5). The HBV viral envelope requires cholesterol for proper infection of naïve cells (6), and more recently a dependence on caveolin-1 (located within plasma membrane caveolae) for cellular entry has been established (7).

The clinical use of cholesterol-lowering statins [inhibitors of 3-hydroxy-3-methyl-glutaryl (HMG) CoA reductase] to treat viral infections is limited and has been tested against HCV with little to no success (8, 9). One study including HIV/HCV-coinfected patients even found an increase in HCV titers following *in vivo* treatment (10), probably because of increased expression of cell-surface receptors [(i.e., LDL receptors (LDLR)], a direct effect of this type of cholesterol inhibition.

Liposomes capable of entering cells for endoplasmic reticulum (ER)-targeted drug delivery have been developed and are superior to other liposome-based systems for the delivery of both hydrophilic and hydrophobic cargo (11). Here we use poly-

unsaturated ER-targeting liposomes (PERLs) in the absence of encapsulated drugs to treat HCV-, HIV-, and HBV-infected target cells and demonstrate a resulting decrease in cholesterol levels within both infected cells and secreted virions for all three viral systems. Lowering cholesterol levels in this manner leads to significant antiviral activity in all three systems and suggests that PERLs may be useful as an *in vivo* therapy to treat a broad range of cholesterol-dependent viral infections and coinfections, either as monotherapy or in encapsulated drug mixtures.

Results

The toxicity of PERLs against cells used for propagation of viral infections was measured, and the highest concentration associated with minimal toxicity was 50 μ M in medium (Fig. S1). At this concentration Huh7.5 cells used for propagation of HCV in cell culture (HCVcc) demonstrated mean decreases in both free and esterified cholesterol of 53% (SD 2.7) ($P < 0.001$) and 25% (SD 1.1) ($P < 0.001$), respectively (Fig. 1A). When treated with 50 μ M PERLs, peripheral blood mononuclear cells (PBMCs) used for HIV assays had a mean decrease of 33% (SD 6.3) ($P < 0.001$) in free cholesterol (Fig. 1B). Similar activity was observed for CD4⁺ T cells (Fig. 1C), the specific subset of PBMCs infected by HIV. HepG2.2.2.15 cells required for HBV propagation and secretion assays showed mean decreases in both free and esterified cholesterol levels of 36% (SD 0.8) ($P = 0.05$) and 54% (SD 0.05) ($P < 0.001$), respectively (Fig. 1D). Reduction in the levels of cellular cholesterol could be a result of sphingomyelinase activation in the presence of increased levels of unsaturated fatty acids within the cells, as suggested by the dose-dependent increase in enzymatic activity observed in all cell lines following treatment (Fig. S2). Lovastatin at nontoxic concentrations was used as a control in both Huh7.5 and HepG2.2.2.15 cells to compare its cholesterol-lowering activity with that of PERLs and was found to be inferior in both cell lines, with no significant effect on sphingomyelinase activity. Lovastatin could not be used in PBMCs because the concentrations necessary for cholesterol inhibition are toxic. A PEGylated version of PERLs [including a PEG-phosphoethanolamine (PE) lipid at a molar concentration of 3%], which increases *in vivo* stability, demonstrated activity similar to that of the non-PEGylated version. Although there is no direct evidence that the ER-targeting capabili-

Author contributions: S.P. and N.Z. designed research; S.P., N.B.N., A.B., and C.R. performed research; S.P., N.B.N., R.A.D., and N.Z. analyzed data; and S.P. wrote the paper.

Conflict of interest statement: R.A.D. is a Director and Member of the Scientific Board of United Therapeutics Corp., which supported this work in part through a "Blue Skies" research grant. A patent covering cholesterol level-lowering ER-targeting liposomes has been filed.

Freely available online through the PNAS open access option.

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