40 Years Ago

Gordon Research Conference on Immuno-electron microscopy in 1972: A tipping point for immuno-enzyme technology

Gwen Childs, Ph.D., FAAA (formerly Gwen C. Moriarty),
Childsgwenv@uams.edu.
Department of Neurobiology and Developmental Sciences
University of Arkansas for Medical Sciences
Little Rock, AR 72205

The first and only Gordon Research conference on Immuno-electron microscopy (EM) was held at Wayland Academy, Beaver Dam, Wisconsin, 40 years ago, July 31—August 4, 1972. Dr. Sydney Breese chaired the conference. I was a brand new Ph.D. having just defended my dissertation May 2, 1972. I was privileged to attend as the guest of Dr. Ludwig Sternberger. My name at that time was Gwen Childs Moriarty.

To set the stage, I need to give you a view of what was going on during the previous decade. The earliest and most notable successes in immune-EM were with conjugates of antibody and ferritin first reported by Professor Seymour Jonathan Singer in 1959 (Singer, 1959). The immunoferritin approach was still growing, and probably most useful for surface antigens, because it penetrated tissue poorly and added non-specifically to plastic ultrathin sections. In 1970, my advisor, Dr. N.S. Halmi had suggested I try the immunoferritin approach to detect adrenocorticotropin in the pituitary for my dissertation research, however, after I read some of the literature on the latest technology with immunoperoxidase, I convinced him to try this approach.

The immunoperoxidase approaches were promising because they allowed detection at both light and EM levels. The pioneering studies by Graham and Karnovsky in 1966 on the ultrastructural detection of peroxidase paved the way for this development (Graham and Karnovsky, 1966) and led to two reports of successful immunoenzyme labeling during 1966. One of the groups, led by Stratis Avrameas at the Pasteur Institute in Paris described the protocol for the conjugation of enzyme to antibody and its application at the EM level, in 1966 (Avrameas and Uriel, 1966; Bouteille and Avrameas, 1967). He developed conjugates of peroxidase and anti-IgG with the use of glutaraldehyde as the bifunctional reagent. At the same time, Dr. Paul Nakane, a young Assistant Professor working with GB Pierce (Ram and Pierce, 1966) at the University of Michigan, used conjugates of peroxidase and anti-IgG were made with a bifunctional reagent, p,p'-difluoro-m,m'-dinitrodiphenyl sulfone (FNPS) and the technique was published in a letter to the editor in 1966 (Nakane and Pierce, 1966), with a full description of the technology published the following year (Nakane and Pierce, 1967), including its application at the EM level.

There were challenges with the early conjugates of peroxidase and antibody in that it was difficult to get a high yield of conjugated antibody and separate the unlabeled antibody. This drove the development of different approaches in three different laboratories. These groups used anti-peroxidase antibodies to attach the peroxidase to the complex, in a bridge, taking advantage of the bivalent properties of IgG binding. Dr. Ludwig Sternberger, a Professor at Johns Hopkins University and also at Edgewood Arsenal, Maryland, referred to the approach as the "unlabeled antibody method" (Sternberger LA, 1969) whereas Dr. Samuel Spicer from the Medical University of South Carolina called the protocol the "immunoglobulin-enzyme bridge" method (Mason et al., 1969). Dr. Avrameas called his approach the "mixed antibody method" (Avrameas, 1969; Avrameas, 1970). The big challenge with this method involved the elution of high-affinity anti-peroxidase molecules. Often the highest affinity molecules remained on the column. So, Dr. Sternberger developed the technology one step further with the peroxidase-antiperoxidase (PAP) technique (Sternberger et al., 1970) and essentially solved the problem of elution of anti-peroxidase. In this sequence, the third antibody to peroxidase is added in a complex with peroxidase (PAP complex), which is soluble and brings 3 peroxidase molecules to the antigen-antibody sequence. The result was a publication that became a citation classic in 1983, with over 1280 citations. Today, the Web of Science lists over 5,799 citations for this landmark paper. After trials with the conjugate, I used PAP for my dissertation work and the strong labeling with relatively low background surprised everyone, including me (Moriarty and Halmi, 1972). It really showed that the immunoenzyme technology was extremely sensitive and feasible for both light and EM. Thus, the stage was set for a highly informative Gordon Research Conference in 1972.

The 1972 Schedule for Gordon Research Conferences carried the theme "Frontiers of Science" and the program broadly covered all aspects of the field, although the use of colloidal gold was notably absent. Faulk and Taylor had just published their landmark paper (Faulk and Taylor, 1971) and unfortunately they were not represented.

It began, July 31, 1972 with a lively discussion of "Protein Chemistry of Antibody Labeling" with Drs. Nakane and Sternberger as speakers. Dr. Nakane was discussion leader. The second session was "Immune-Ferritin Preparation" by Drs. Konrad C Hsu, Columbia University; Arnold Vogt, Hygiene-Institut der Universitat; and Sidney S. Breese (Plum Island Animal Disease Lab and the Chairman of the conference). Tuesday, August 1, was devoted to "Immuno-ferritin (viral applications)" by Drs. Coulcilman Morgan, Columbia University and Calderon Howe, Louisiana State University and "Immuno-Ferritin (tissue applications)" with Drs. Samuel Dales, Public Health Research Institute of New York, and Margaret J. Polley, Cornell University Medical College. Wednesday morning, there was an "Immuno-Ferritin" workshop led by Drs. Sidney Breese, KC Hsu, and Guiseppe Andres, Medical School State University of New York.

On the afternoon of August 2, "Immune-enzyme techniques (peroxidase)" were discussed, led by Dr. Stratis Avrameas, Institute Recherches sur le Cancer as discussion leader, and speakers Drs Ph.J Hoedemaeker, Dept Pathology, Groningen, and Robert L

Vernier, University of Minnesota. Dr. JP Kraehenbuhl, Rockefeller University then discussed the pioneering use of cytochrome as an enzyme marker. Thursday, August 3 involved Dr. Nakane's "Immuno-enzyme Technique Workshop" with Dr. Guiseppe Andres, and a "Hybrid Antibody Technique" presentation by Drs. T Aoki, National Cancer Institute and Christopher Stackpole, Sloan Kettering Institute for Cancer Research.

Finally, on Friday, August 5, half of the morning session was devoted to the "Unlabeled Antibody Enzyme technique" with Dr. Ludwig Sternberger. The remaining half ended with a discussion of "Immunizing markers and tracers" led by Dr. Stratis Avrameas.

As you can tell, the time-honored immunoferritin approaches received top billing as they had been around for 13 years. The second approach that was emphasized was the immunoperoxidase conjugate techniques. Dr. Sternberger had been invited to present his newest "unlabeled antibody methods" and he invited me to present my dissertation research photos during that session, showing how well the PAP complex worked.

I can state that the opportunity to meet and listen to so many pioneers in this field was enriching and very empowering. I was probably not prepared for the level of discourse, challenges and debating. All throughout the conference, the immunoperoxidase technology was greeted with many questions. Dr. Sternberger was the sole representative of the newest "unlabeled antibody approach". He did not hesitate to challenge and question each of the speakers all week long. Some of the participants learned about our results with the PAP complex and asked for an early mini-workshop during the meeting. We accommodated whoever wanted to attend this informal session, on the side.

When it came time for Dr. Sternberger's formal session, the participants were more than ready to return the favor of debating and questioning. Of course, because I presented my dissertation work during that session, this was a true baptism by fire for a young Ph.D. These scientists were a tough crowd; the data were greeted with appropriate skeptism and many questions and challenges, some quite daunting.

I kept a stiff upper lip, as I recall, but felt that I must have failed miserably in convincing them about our work. At the airport later that day, I heard my name being called from the bar and a group of the toughest questioners invited me to join them for a beer. This was also a revelation! Immediately I recognized that I had just joined the club of fellow researchers who could challenge one another one minute and celebrate, collegially, with a beer the next. Sometime later, when corresponding with Dr. James Jamieson, (who was at Rockefeller University at that time and one of the more active questioners), he apologized saying "I realize my comments to you may have come out sounding harsh or hypercritical. They were not meant to be, but if so, I am sorry. I must say that your work was very impressive and in my opinion the best shown at the meeting. Your pictures were truly gorgeous and I want to congratulate you on your results."

Figure 21 is a photograph of the participants in this conference and we have appended names and a key. I believe that the Conference was clearly a tipping point for the field as it showed how well immunolabeling had progressed. I recently sent out the

photo to some of the conferees and asked for their recollections. I received a wonderful response from Stratis Avrameas. I will share this from him.

"Thank you for your e-mail. Unfortunately I don't remember much things from that Gordon Conference meeting although I remember that I have read, following that meeting ,several published articles by G.Moriarty. I am also almost sure that Coons and Leduc were not present.

The only thing I certainly remember, because I met it during all my scientific life, was the interest, but at the same time, the high skepticism with which was received the new, at that time, field of immunoenzymatic technology. It came to my mind that the ideas of Bertrand Russell, who I was reading at that time, applied equally well to religion and science. That is, with my own words, "A new truth disturbs especially the authority. However all the long history of hardness and intolerance, it is the highest acquisition of our clever but stubborn human species". Stratis Avrameas, personal communication, July 8, 2012.

I invite members of the society, who may have participated, or whose mentor may have participated to send me their recollections of this meeting (Childsgwenv@uams.edu). I am writing a chapter on the history of immunocytochemistry and it would be great to have additional input from other participants!

If you follow publications from the participants, you can tell that this conference and the publications in 1972-73 eventually led to some of the best early electron and light microscopic immunolabeling, stemming from advances in the EM protocols. During the subsequent decade, I found myself in many training sessions by phone or letters helping people with their protocols. I often wonder today how having email would have improved communication. It has been gratifying to watch the growth of the field in both cellular endocrinology and neuroscience thanks to these early immunolabeling advances.

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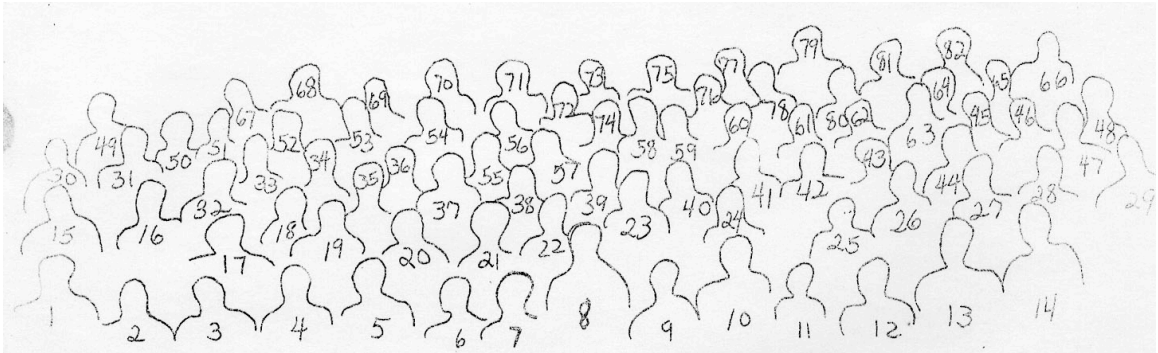
GORDON RESEARCH CONFERENCES
IMMUNO-ELECTRON MICROSCOPY
JULY 31 – AUGUST 4, 1972
WAYLAND ACADEMY
BEAVER DAM, WISCONSIN
MR. SYDNEY S. BREESE, JR., CHAIRMAN



GORDON RESEARCH CONFERENCES

Conference on Immuno-Electron Microscopy

Wayland Academy
Beaver Dam, Wisconsin
July 31 - August 4, 1972

SYDNEY S. BREESE, JR., Chairman

1. John W
2. Dan Raveed
3. Jesus M. Vazques
4. Stratis Avrameas
5. Arnold Vogt
6. Christopher C.K. Leung
7. Paul K. Nakane
8. Sydney Breese
9. Konrad C. Hsu
10. G.A. Andres
11. Beatrice Seegal
12. Ikuko Nagatsu
13. Councilman Morgan
14. Philip Hoedemaeker
15. Andrew Engel
16. C. Neauport
17. Thomas J. Jacks
18. Sheila Tannenbaum
19. Walter H. Lewis
20. Joel Warren
21. Manuel S. Valenzuela
22. Mildred Gordon
23. I. Molenaar
24. Esther Palmer
25. A. Goldfeder
26. Sue Sisson
27. Robert L. Vernier
28. Rodrigo Urizar
29. Bert C. Frichot III
30. Gerald Goldstein
31. J.T. Stasny
32. M. Tannenbaum
33. S. Dales
34. Jay C. Huang
35. R.E. Williams
36. N. Baba
37. Wolf Kuhlmann
38. Gerard Feldman
39. David J. Silverman
40. Sharon Collins
41. Ludwig Sternberger
42. Gwen C. Moriarty
43. Ida Tenenbaum
44. Harry B. Neustein
45. Arthur Katoh
46. William H. Zucker
47. Larry F. Lemanski
48. V. Herzog
49. Jan R. Brentjens
50. Lidia Accinni
P.E. Bigazzi
52. F.M. Kourilsky
53. Wm. Hooker
54. Leon Hoyer
55. J.P. Kraehenbuhl
56. Arlis D. Boothe
57. Sharon Whitaker
58. Richard Rodewald
59. Harold H. Edwards
60. Marcus Ma
61. Shao-Nan Huang
62. Michael Gerber
63. J. Perrotto
64. E. Phillips
65. H. Dembitzer
66. Milas Boublik
67. C. Howe
68. Karl Fresen
69. Al Ritchie
70. David Stage
71. Dickson Despommier
72. Mart Mannik
73. Richard Foemmel
74. Madan Varma
75. Robert H. Gray

76. G.B. Bouck

77. Michael A. Clark

78. P. Biberfeld

79. Sterling Ainsworth

80. J. Jamieson

81. Margaret Polley

82. E.A. Zimmerman