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The *S. japonicum*-Based pGEX Vector: Commercial Outcomes from Analysis of Model Host-Parasite Relationships in a "North-South" Collaboration

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Abstract: As judged by widespread utility in protein production from recombinant *Escherichia coli* and by the magnitude of royalty payments to Melbourne's Walter & Eliza Hall Institute (WEHI), the expression vector pGEX, invented by Dr Donald Smith, has been a significant commercial success. It is based on the 26kDa glutathione S-transferase of *Schistosoma japonicum* (Philippines) termed Sj26GST, that emerged from work throughout the 1980's on resistance to infection in a peculiar mouse strain, WEHI 129/J. Sj26GST was the lead vaccine candidate for this human helminth worm being pursued in a long-term collaboration between WEHI in Australia and Dr Edito Garcia's[1] group at the College of Public Health, University of the Philippines in Manila that commenced in 1980.

The product, pGEX, is an excellent example of commercial spin-off from basic research in mouse model systems that indeed evolved into an applied research program but with a very different goal, namely rational molecular vaccine development.

INTRODUCTION

In 1980, a collaboration was initiated between Dr Edito Garcia's laboratory at the Institute (later, College) of Public Health, University of the Philippines, Ermita, Manila Philippines and Dr Graham Mitchell's laboratory/Unit at The Walter and Eliza Hall Institute of Medical Research (WEHI), Melbourne, Australia. This followed a visit to Manila by Mitchell to attend a WHO-sponsored conference on human trematode parasites (*Paragonimus*, *Clonorchis*, etc). The WEHI Immunoparasitology Program headed by Mitchell did research on a variety of protozoan and helminth parasites in mice of various genotypes and immunological status to study immune responses to parasites. This was in addition to broad analysis of susceptibility and resistance to infection or disease. What the program lacked was a helminth infection of major medical (as distinct from veterinary) significance. A chance afternoon visit during the conference to Dr Garcia's laboratory immediately provided the opportunity of working with *Schistosoma japonicum* (Philippines). The Manila laboratory had a large "snail room" with breeding *Oncomelania* snails and the life cycle going in rabbits and mice. Additionally, human material was available through routine serological diagnosis of schistosomiasis japonica using the circumoval precipitin test (COPT).

With funds from Ken Warren's Rockefeller Foundation Great Neglected Diseases Program, the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR Program, Geneva) and the Australian National Health and Medical Research Council (NH MRC), a 15- year "North-South" collaboration was initiated. This long-term program on schistosomiasis combined parasitology and public health in the Manila laboratory; immunology, immunogenetics, immunochemistry and molecular biology in Melbourne; and field studies in Irosin, Sorsogon, a Field Station having been constructed with funds from the Australian Development Assistance Bureau, Canberra (ADAB).[2]

During this collaboration, the important concept of anti-embryonation immunity was developed in Manila – ie disease amelioration in chronic schistosomiasis japonica results from embryocidal immune responses, reduced antigen production from the egg, and thus reduced granulomatous disease and liver pathology. We speculated that disease modulation had less to do with immune modulation and more to do with inhibition of production of antigens from the developing miracidium within the egg and lodged in tissues [1-3].

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[1]The authors are privileged to dedicate this article to our collaborator, mentor and generous friend, Dr Edito Garcia; it is written to commemorate the 100 year anniversary of the discovery of *Schistosoma japonicum* in the Philippines in 1906.

[2]The long-term commitment by both parties to the Australian-Philippines collaborative venture came about not only because of the immediate productivity and rapport that developed, but also from a conversation with the Mayor of Irosin when we were negotiating access to land owned by a Dr Mateo to build the Field Station. Mayor Dorotan referred, in a very Philippine way, to the evils of what is commonly known as "safari research" – riding into town with much fanfare and quick talking, collecting samples and disappearing back to Manila and Melbourne! He referred to this type of activity as "ningas cogon" – this being a type of grass that grows rapidly in the wet season and, when dry, burns with much flame, smoke, sparks and noise, but leaves virtually no residue (by way of ash in this case)!! We gave him a 10-year commitment on the spot and thanked him for his wisdom.

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Another finding was just how different the Philippine strain of *S. japonicum* was from other schistosomes in many behavioural characteristics in mice, particularly lung migration [4].

INITIAL EXPERIMENTS – IDENTIFICATION OF A RESISTANT MOUSE STRAIN

Mice of various inbred strains were transported from Melbourne to Manila where they were exposed to *S. japonicum* cercariae. All mouse strains were "permissive" hosts except for 129/J [5-8]. This observation – precisely what we were looking for – led to an intensive effort to identify immune responses (ie antibody specificities) in the sera of resistant 129/J mice that differed from these in susceptible strains such as BALB/c, CBA/H, C57Bl/6 etc. Much use was made of 1D and 2D gel analysis of immunoprecipitates of radiolabelled worm extracts as well as, later, Western blotting [9].

A SEROLOGICAL CORRELATE OF RESISTANCE-ANTI SJ26 ANTIBODIES

In the hands of a senior technician, Ms Kathy Davern, superb quality ID and 2D gel analyses demonstrated that exposed, resistant 129/J mice consistently recognised a series of apparently related molecules in the Mr26,000 (26kDa) region of the gels relative to chronically-infected (genetically-susceptible) mouse strains [10-11]. This, again, was precisely the sort of result we sought! We reasoned that if susceptible strains of mice could be appropriately vaccinated and mount immune responses (antibodies?) to this molecule or molecules, then resistance to subsequent infection may result. We certainly believed we were hot on the trail of the first molecular vaccine against schistosomiasis. Studies with human sera quickly established that humans could respond, albeit weakly, to antigen(s) in the 26kDa region of gels [11-13]. We termed the antigen(s) Sj26.

IDENTIFICATION OF SJ26

Dr Rob Saint constructed an adult worm cDNA expression library of *S. japonicum* antigens in *E.coli* using λgtII [14]. Identification of any colonies producing Sj26 was problematic simply because we had particular difficulty in developing anti Sj26 antibody probes for screening the library. In a vivid demonstration of the need to "heed the advice of the Laboratory Head selectively", another senior technician, Ms Jacqui Beall, suggested we elute antibodies off the 26kDa region of Western blots, pool the antibodies and use them to probe the expression library. Told not to waste our time with stupid suggestions that could not possibly yield enough antibody, Jacqui went away and performed the experiment anyway! In very rapid time we had 4 positive clones and a technical hurdle instantly overcome [15]. A sequencing effort headed by a post-doctoral fellow, Dr Donald Smith, and involving Kathy Davern and others, identified Sj26 as a glutathione S-transferase (GST) [16-17].

THE PGEX INVENTION

With identification of Sj26 as a GST (Sj26GST) we reasoned that antibody neutralisation of an enzyme that may have detoxification and protective functions for the worm, could render it susceptible to other immune responses induced during infection. In other words, a molecular vaccine directed to a "functional antigen" of *S. japonicum* seemed within our grasp.

Dr Phil Board of the John Curtin School of Medical Research, Canberra, alerted us to the fact that glutathione could be used to affinity purify the Sj26GST. Vaccination studies in mice that had commenced using crude antigen preparations from *E.coli* cultures (indeed, fusion proteins of β-galactosidase) could be designed using purified Sj26GST [16, 18-20].

Whilst vaccination studies were in progress (and yielding modest protective effects at best and quite inconsistent between experiments), Donald Smith pursued the idea of using the cloned Sj26 in an expression vector to produce a fusion protein. This would comprise a protein of interest tagged with Sj26 that could be purified on a glutathione column and subsequently eluted. Dr Smith and colleagues invested a large effort in developing, modifying and optimising the expression vector system [17, 21].

COMMERCIALISATION

In Melbourne at about that time, the unlisted biotech company, AMRAD, was established by the Victorian State Government with WEHI as a major shareholder. The new CEO, Dr John Stocker, a former PhD student at WEHI, had been recruited back to Australia after 10 years with Hoffman La Roche in Basel, Switzerland. Following discussions between Mitchell and Stocker, AMRAD licensed the technology from WEHI and developed its first commercial product – Glutagene [22] later named pGEX. This expression vector that produces a readily-purified, *E.coli*-derived fusion protein containing the 26kDa glutathione S-transferase of *Schistosoma japonicum* (Philippines) has found wide utility in research and in protein production. It has earned WEHI in excess of A$2.2m in royalty payments since the late 1980s – the Australian launch occurred in April 1988. To quote [22], "…Glutagene derives from basic research and the quest for a molecular vaccine against schistosomiasis. …..The story of the background of Glutagene illustrates a universal feature of biomedical research, namely the collaboration of many individuals. …..Nothing could illustrate better the multiplicity of technical, intellectual and financial inputs that sustain basic research and from which unexpected commercial spin-offs emerge".

CONCLUDING COMMENT

As we have outlined in this brief article, the commercially-successful, widely-utilised bacterial expression vector, pGEX, resulted from the detailed analysis of the immunology of a model host-parasite relationship and subsequent quest for a model schistosomiasis vaccine. It was not remotely anticipated when the Manila-Melbourne collaborative program was initiated a mere 7 years earlier.[3]

[3]As an aside, we have been honoured to hear both the Rockefeller Foundation and the TDR Program refer to this long-term program as a "model" of North-South (perhaps "South-North"!) collaboration. It has certainly been a most pleasurable experience for us and we are grateful for high-level, long-term financial support and a very large number of dedicated, talented and convivial collaborators.

To return to the 129/J mouse strain at WEHI in the 1980s, subsequent studies indicated that anti-Sj26GST antibodies are in all probability totally unrelated to resistance! In an ironic twist, we demonstrated severe portal system changes in WEHI 129/J mice such that resistance could be readily ascribed to a failure of worms to access or remain in their preferred locations, namely the mesenteric veins and liver. Rather, they accumulated and presumably died in the lungs of these mice [7-8, 23-24]. Release of antigen from dead and dying worms (versus healthy worms in permissive mouse strains) presumably leads to increased titres of anti-Sj26GST antibodies, 129/J also being a genetic high responder [25].

WEHI 129/J mice were very different from 129/J mice purchased from the Jackson Laboratories and exposed to *S. japonicum* cercariae without long-term residency in WEHI mouse rooms. We postulated though could never prove a nutritional contribution (? Vitamin A toxicity) to liver pathology and portal system changes. We learnt that a serological correlate of resistance is just that, a correlate, and resistance may have nothing to do with immunology. A sobering lesson for immunologists although we hasten to say we were always cautious in interpretation of anti-Sj26 responsiveness in resistant mice! [10].

In regard to the vaccinating efficacy of Sj26GST or for that matter the related 28kDa GST molecule, Sj28GST [26-27], we were never able to consistently demonstrate increased resistance to *S. japonicum* in vaccinated mice despite rather heroic attempts [19]. The 28kDa GST of schistosomes is a candidate (anti-fecundity) vaccine for schistosomiasis mansoni, now in advanced clinical trials and developed in France by Dr André Capron and colleagues. Sm28GST, unlike Sj26GST, was first identified using monoclonal antibodies with inhibitory effects on *S. mansoni*. Time will tell whether the GSTs of schistosomes can be developed as useful affordable vaccines in their own right or, more likely, as adjuncts to chemotherapy [28] and in a multi-component format with appropriate adjuvant.

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