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EDITORIAL

Targets and Tools of Trade: Immunotechnology and Immunoeconomics

From the perspective of Landsteiner looking forward to the present, immunology's advance would seem impressive, perhaps even relentless. From the present looking back to Landsteiner the perspective is equally amazing, for his delineation of the elements of antigenic specificity using small molecular weight chemical isomers stands as a dynamic monument to molecular insight and ingenuity, in many respects way ahead of his time. Between the two boundaries of this kaleidoscope of time lies a historical drama of dialectics, often semantic in nature, where cellular theory was periodically compelled to wait for evolution of technology to permit molecular determination of protracted debates. Despite persisting and multifarious untied ends, the genetic jigsaw of the immune system now contains many molecular monuments in its expanding archives, such as immunoglobulin structure, the mechanism of antibody diversity, the structure and diversity of T cell receptors, the cytokine family, MHC restriction and so forth.

At the more pragmatic end of the spectrum the era of monoclonal antibodies [1] is already golden, with the advent of antibody engineering [2] as an exciting sequel. This latter development underscores the pluripotency of recombinant DNA technology as applied to the immunological arena, since it has for some years represented a means of providing defined antigens and is contributing extensively to immunogen design [3] for new vaccine development. The applications of monoclonal antibodies to immunodiagnosis are widespread and diverse in nature, the key advantages being specificity, quantity, and quality control. Their potential in immunotherapeutic targeting is well recognized but still somewhat elusive in practice. They represent a paradigm of precise molecular tools for molecular targets.

Delineation of the precise epitopes targeted by both antibodies [4] and by T cells [5] has raced ahead as a game of greater and greater molecular precision over the past several years. This advance has rested on simpler and faster methods of defining protein structures at the primary, secondary, tertiary and quaternary levels, [6] so that at least for some antigenic proteins we now have fine detail of antibody and/or T cell binding sites in terms both of amino acid sequence and of topography. [7]

The unfolding molecular landscape detracts not one iota from the intrinsic mystery and beauty of past dreaming about the origins and nature of the complexity of the immune response. As each small piece of the jigsaw is identified and intertwined with its partners broader segments of the total masterpiece emerge from the haze of history. Reductionism has its creative role in the holistic synthesis.

But there are urgent and compelling pleas to descend from the mountain to the semi-arid plains below. From one viewpoint modern immunology has already contributed greatly to the practical world of diagnostics, disease monitoring and therapeutics. In this process, however, one major driving force is the lure of hi-tech medicine and its rewards in the private clinics or large teaching hospitals of the metropolis. There the laboratories abound in immunodiagnostics for infectious, allergic and chronic diseases. There is increasing precision, increasing confidence and increasing speed : applied immunology can rightly expect acclaim as a success story in the practical world of clinical medicine, particularly in centers of ultimate referral in the cities, but also in the suburbs.

From another viewpoint there is still a long way to go, a major

challenge still ahead. Immunoeconomics is a science already born, perhaps a trifle premature but waiting to be nurtured into a more certain childhood; some distance, however, from the threshold of adulthood. In the early 1970s at NIH I remember listening to Alistair Voller extoll the virtues of ELISA plate assays, incubated in plastic bags under the sun, for assessing causes of diarrheal disease in Bangladesh : it was an exciting, refreshingly different view of immunotechnology from one very much attuned to the real world. Time has reinforced and expanded the pre-eminent place of ELISA systems of many kinds in diagnostic immunology world-wide. But despite being simple in concept and offering quantitative precision, ELISA still represents technology which is too complex or too costly for routine use where most disease occurs, in the villages of the world. Conversion to dot ELISA assays [8] is making great strides to close the complexity gap, especially with the substitution of enzyme indicators by single-step immunogold methods [9] but the economic equations are still somewhat disheartening, sensitivity is often less and quantitation difficult.

This presents a challenge to motivation, to economic rationalization and above all to immunotechnology. The objectives are clear : simpler, cheaper probes for infectious, allergic and chronic diseases which can be used at the periphery without costly machines or highly trained personnel; tools for disease diagnosis, disease monitoring, disease control and disease surveillance, as well as tools for mechanistic analysis which themselves may later generate improved methods of disease management at the periphery. Speed is of essence, as is the continuing improvement of disease management and monitoring strategies with consequent increased efficiency in information transfer to and from epidemiologic files.

Strategy modification is a true handmaiden of technology advance. Let us take one example. Malaria case detection (active and passive) and drug treatment can be an effective control strategy if it encompasses most cases in a region. Malaria case detection presently depends on classical microscopy but this is often suboptimally effective at the peripheral clinic level because of limited microscope availability, the high degree of technician training required and the time involved. For these reasons clinical diagnosis and presumptive therapy are a common *modus operandi* in many endemic areas: at best this covers overt clinical cases but ignores the asymptomatic reservoir. Logistics and time delays in microscope slide referral to central laboratories are prohibitive for individual patient management, useful though they are for epidemiologic assessment. DNA probes, exquisite though they are in specificity, [10] have similar disadvantages. Plate ELISAs are also central laboratory tools, whether they measure antigen or antibody. One minute, single step agglutination or dot assays using whole finger prick blood are the required targets: moderate false *positive* rates would be acceptable for a rapid screening strategy if light microscopy or fluorescence microscopy [11] is retained as a *selective* confirmatory and quality control measure. At the periphery it may then be permissible to exclude parasite species identification as a requirement in the initial immunoassay, giving technology development a much freer hand and greater chance of success. Analogous arguments can be put forward for handling a number of infectious diseases at the village or district level.

Advances in technology give reason for cautious optimism that such objectives are now approachable, if they are the clearly focused targets of new developments. Thus, *broad* rather than narrow monoclonal antibody specificities, based on flexible rather than rigid antigen combining sites [12] can be advantageous as tools to capture a range of species or strains of virus, bacterium or parasite causing a particular disease class. Such a proposal goes against conventional wisdom which idealizes the exact identification of the right needle in the haystack, but it allows speedy recognition that there is a particular sort of needle present. Such promiscuous monoclonal antibodies can be deliberately sought. The target of engineered, [2] stable, broad specificity monoclonal antibody fragments for such screening purposes is within the realms of the possible, potentially at reasonable downstream cost and with high quality control. The finesse of applying species-specific, strain-specific or even mutant-specific immunoprobes would then be more of a central laboratory job, amenable to current molecular technology but not so cheap.

Another relatively new horizon has arisen that gives real hope and expectation. The ability to scan whole protein antigen sequences per synthesis of hundreds or even thousands of overlapping oligopeptides in a few days [13] has totally changed the game of T and B cell epitope analysis. [14,15] All *linear* epitopes can now be defined for any protein antigen for which the nucleotide sequence and derived amino acid sequence is known. [16] This leads to the synthesis of precise target oligopeptides for antibody [17] or reactive T cell [14] detection assays or to precise peptide immunogens for targeted, site-directed monoclonal antibody production. Once the selection has been made in this way, conventional synthesis of the target peptide in quantity represents a trivial proportion of the total cost of an assay kit, thus placing the economic selection pressure on the process of simplified assay development itself. Most small synthetic peptides are also more stable than cruder or larger (e.g. recombinant) antigens, leading to longer potential assay shelf-life.

Given this quantum leap in the capacity to produce defined molecular reagents, the onus now is on rapid test format design. Two prototypes are already available. Immunogold-based dot assays using synthetic peptides have promise in terms of speed but at present require serum separation, preferably by centrifugation. Agglutination assays of the AGEN [18] type based on whole blood with red cells as the solid phase and a single peptide-monoclonal antibody reagent have promise as one minute field assays: modifications of this concept are eminently conceivable. Both prototypes are qualitative or at best semi-quantitative, both are at present aimed at antibody detection but could in principle be developed for antigen detection. However, they do represent examples of immunoassays which can be usefully handled in the small field clinic by virtue of speed, reagent stability, simplicity and potentially low cost. More importantly, what these innovations do is to open the doors of imagination to a variety of ways in which current monoclonal antibody and peptide technologies might be applied in the real world of village immunoeconomics.

Even if it be only approximate, more rapid on-site case detection improves case management and provides quicker access to epidemiologic information, which leads inturn of more effective disease control strategies. Allergic diseases are also large in number and of considerable epidemiologic importance. For more rapid, precise and cheap assays of protein allergen hypersensitivity the same peptide technologies offer the possibility of making targeted molecular probes for sequence-defined protein allergens such as those of house dust mites. They should also be able to provide very specific desensitization tools, thus converting one of the older arts of medicine into a more exact science. To bring these options within range of the poor majority of the world requires that the economics of the village ultimately *dictate* the targets of appropriate technology: molecular tools are now close to hand which should be able to make this challenge attainable both technically and economically. Such developments must of necessity rely on quality control provided by more quantitative, more precise central laboratory tools, not seek to replace them.

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