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industry. Most biotech companies in South Korea are small and relatively immature. If they are to succeed, their best opportunities lie in collaborations with domestic and foreign companies. These will allow them the best mix of economic support and will help reduce the risks involved in drug development.

References

1 Anon (2005). Biotechnology Industry Facts. Biotechnology Industry Organization. http://www.bio.org/
2 Kermani, F. (2004). There's Something About Biotech. Contract Pharma magazine. October 2004 issue. http://www.contractpharma.com
3 Anon (1998). South Korea: economic reforms run into difficulty. BBC News. 8th January 1998. http://news.bbc.co.uk/1/hi/special_report/1998/asia_economic_crises/45476.stm
4 Anon (2005). South Korea: Forecast. The Economist Intelligence Unit. http://www.economist.com/countries/SouthKorea/
5 Woods, M. (2003). Europe slow in stemming 'brain drain' to America. October 20, 2003. The interactive version of the Pittsburgh Post Gazette. http://www.post-gazette.com/pg/pp/03293/232608.stm
6 Anon (2004). Hsinchu Science Park. Yearly statistics. Administration of Hsinchu Science Park. http://www.sipa.gov.tw/en/
7 Anon (2003). Biotechnology: Government to Inject 539.3 billion won in Biotech this year. British Embassy, Seoul. Registered: 15th May 2003. http://www.uk.or.kr
8 Anon (2003). Korea Biol. Venture Association (KOBIOVEN). http://eng.kobioven.or.kr
9 Sung-jin, K. (2005). Korea to Inject W260 Billion Into Biotech. 21st February 2005. The Korea Times. http://times.hankooki.com/tech/tech.htm
10 Ahn, C. (2003). Emerging Biotech Market. US Commercial Service. US Embassy Seoul. http://strategis.ic.gc.ca/epic/internet/inimr-ri.nsf/en/gr116479e.html
11 Ahn, C. (2004). Biotechnology Industry in Korea. US Commercial Service. US Embassy Seoul. http://strategis.ic.gc.ca/epic/internet/inimr-ri.nsf/fr/gr117524f.html
12 An overview of the Korean pharmaceutical industry and its R&D activities. Korea Drug Research Association (KDRA). http://www.kdra.or.kr/english.html

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Sourcing a chemical succession for cyclosporin from parasites and human pathogens

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David Pritchard is an immunologist and parasitologist conducting research with the express aim of fully understanding several key host-pathogen relationships. He graduated with a PhD in Immunology from the University of Birmingham, and has industrial, university and tropical research experience.

Several clinical centres worldwide are gearing up to trial parasitic infection for the treatment of asthma and Crohn's disease. The present article takes a step forward, and advocates the mining of pathogens from humans, by the pharmaceutical industry, to source novel small molecule drugs for the treatment of immunological disease. It also argues that judicious source selection will lead to the discovery of discriminatory treatments for the phenotypically distinct immunological diseases of humans, with drugs from bacteria to treat autoimmunity (e.g. type 1 diabetes, inflammatory bowel disease, rheumatoid arthritis), and from nematode parasites to treat allergy and asthma.

Diseases with an immunological basis, allergy, type I diabetes, rheumatoid arthritis, inflammatory bowel diseases and psoriasis, affect hundreds of millions worldwide, yet are poorly served by existing therapies, which are often anti-proliferative broad spectrum immune-suppressants, for example azathioprine, mycophenolate mofetil, and the corticosteroids.

Consequently, these therapies fail to exhibit immunological selectivity, given that they suppress the whole immune system, leading to unwanted side effects. They also tend to target the symptoms of disease as opposed to the underlying clinical pathology of the disease cycle.

Target-specific antibody-based therapeutics, and recombinant cytokine receptors offer alternative routes to treatment, but are associated with the disadvantages of being proteinaceous and expensive, and potentially immunogenic. Therefore, a need remains for novel small molecule therapeutics, particularly with immunological selectivity to allow the discriminatory treatment of diseases with an allergic or autoimmune phenotype (see Intelligent Source Selection below).

The HTS approach to drug development could provide an answer, yet is dependent on the accurate identification of disease-related molecular targets. The molecular complexity

1359-6446/05/$ – see front matter PII: S1359-6446(05)03395-7

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TABLE 1

The biological and geographical sources of commonly used immune suppressive agents

Immune suppressant	Source organism	Geographical source of soil sample
Cyclosporin A (CsA) (Sandoz-Novartis)	*Hypocladium inflatum gams*	Norway
FK506 (Tacrolimus) (Fujisawa)	*Streptomyces tsukubaensis*	Japan
Rapamycin (Sirolimus) (Wyeth-Ayerst)	*Streptomyces hygroscopicus*	Easter Island

of immunological diseases has rendered the associated molecular targets elusive in many cases. For example, in an immunologically multi-faceted disease such as asthma, it is difficult to home in on molecular targets involved at the initiation of the disease process.

As an alternative approach, this article proposes the exploitation of parasites and human pathogens for the discovery of novel chemical leads for immunological diseases, the aetiology of which is inexorably linked to host protective immune responses. The parasites and pathogens have selectively unlocked these immunological processes by evolving as yet poorly understood immune selective and hence disease-selective chemical evasion strategies. Understanding this chemistry will in turn lead to a clearer understanding of immunological activation pathways, and identify new targets for therapeutic intervention. Ironically, our current and partial knowledge of these signalling pathways stems from the early and serendipitous discovery of the currently used repertoire of immune suppressants from the soil microorganisms highlighted in Table 1. This unlikely sourcing of immune suppressive compounds, from soil yet active in man, led to one of the major immunological discovery milestones to date, the beginning of our understanding of T cell activation pathways and inflammatory processes.

Early successes in immune suppression and new initiatives

The discovery of the immune suppressive compounds cyclosporin, FK506 and rapamycin revolutionised transplantation medicine, immune therapy and our knowledge of T-cell biochemistry [1–3].

Their discovery led in turn to the isolation and identification of their immunophilin molecular targets, using immobilized compounds and affinity chromatography. This enabled molecular immunologists to link T cell surface receptor mediated activation events to downstream pro-inflammatory or host-protective cytokine production via the immunophilin biochemical intermediary [4,5]. This is a remarkable scientific development when one reconsiders the unlikely source of these now widely used compounds and represents the pinnacle of 50 years of immune suppressive drug discovery [6].

The role of serendipity in this key immunological and medical development is self evident. Nevertheless, given the historical success of this discovery process, several initiatives have been established recently to mine sometimes exotic source organisms for new leads, in a race to develop new, less toxic therapeutics for immunological diseases and to service transplantation medicine. Sources include the actinomycetes, marine organisms, plants and insects [7–10]. However, this could represent a 'needle in the haystack' approach, in comparison with the strategy outlined in this article, which carefully considers the aetiology of immunological diseases, and the close biochemical and molecular link between pathogens of humans and their immune system.

Consequently, it is possible that the pharmaceutical industry is missing an opportunity to discover new therapeutics by failing to identify more-rational sources of immune modulatory agents. After all, the immune system evolved in a compartmentalized manner to protect the body from infectious disease. These same compartments now cause immunological disease, when overactivated and targeted to autoantigens and allergens (at its most simplistic, T helper 1 > antibacterial > autoimmunity; T helper 2 > anti worm > allergy). These lineages can in turn be regulated by T cell populations producing anti-inflammatory cytokines (T reg) [11]. Therefore, by selecting the correct pathogen and its appropriate immunological target, the industry has the capacity to isolate novel, disease and pathway specific small molecule therapeutics either targeting T helper 1 or T helper 2 disease related pathways, or molecules which engage and stimulate regulatory T cell networks.

Intelligent source selection

Here, I propose that the pharmaceutical industry seriously considers mining the metabolomes of several judiciously selected pathogenic organisms, on the premise that these organisms evolved to co-exist with an activated immune system, and therefore produce finely honed and non-toxic immune suppressive compounds to promote their

TABLE 2

Examples of organisms considered for the rational sourcing of immune suppressive compounds[a]

Organism	Evidence for the production of immune modulants
Pseudomonas aeruginosa	Chemical suppression of T cells [12,13]
Yersinia *species*	Subvert macrophages and dendritic cells from intracellular niche [14,15]
Bacillus anthracis[a]	Inactivates alveolar macrophages [16]
Brucella spp	Coexists with phagocytes [17]
Burkholderia spp	Survives within phagocytic cells [18]. Quorum sensing active [19]
Mycobacteria	Compromised macrophage function. Suppresses signalling (ERK1/2) [20]
Fungi	*Candida albicans* – delayed macrophage phosphorylation of MEK, ERK-1, S6 kinases [21]
Heligmosomoides polygyrus	Suppresses T cell function and graft rejection. Anti-allergic [22]
Necator americanus	Apoptosis in activated human T cells [23]. Alleviates community allergy [24]
Trichuris sp	Moderates inflammatory bowel disease [25]

[a]Ideally, the immune suppressive compounds isolated will be small chemical entities, which modify signalling responses, not toxins that kill cells or enzymes that interfere with humoral immunity (R. Titball, pers. commun.).

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survival, reducing the compound attrition rate from bench to clinic.

Although this might represent a major leap of faith by the industry, it is financially and technologically well placed to harness the chemical potential of these organisms to develop novel therapeutics for immunological diseases. In addition, a complete understanding of the virulence determinants of these organisms will enhance our knowledge of their disease profiles, and lead to the potential discovery of the Achilles heel of damaging infectious organisms. The judicious choice of organisms is key to this novel discovery process. As part of an initiative to 'mine' the metabolomes of pathogenic organisms for new compounds, several biological sources have been identified here for initial profiling (Table 2) [12–25].

These organisms have been selected because of their inherent abilities to modulate the human immune system, establishing chronic and often cryptic infection in the process. In addition, the selected panel includes bacterial and nematode pathogens. Bacteria might be particularly attuned to suppressing the T helper 1 responses, which evolved to combat bacterial infection, but which also cause autoimmune disease. Nematodes are attuned to suppressing the T helper 2 responses, which evolved to combat helminth infections but which also underpin the pathogenesis of allergic diseases such as asthma. Other bacteria and parasites can engage regulatory T cell networks selectively [26].

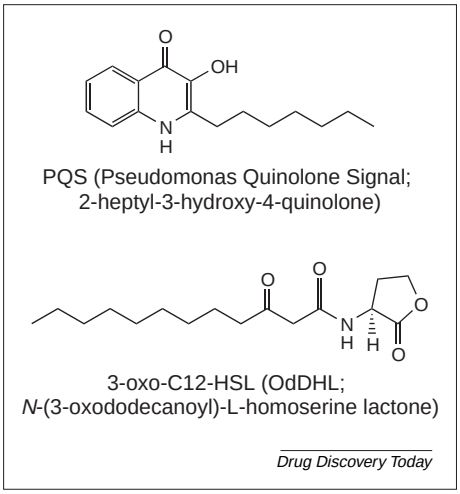

FIGURE 1

Chemical structures of *Pseudomonas aeruginosa* quorum-sensing signalling molecules.

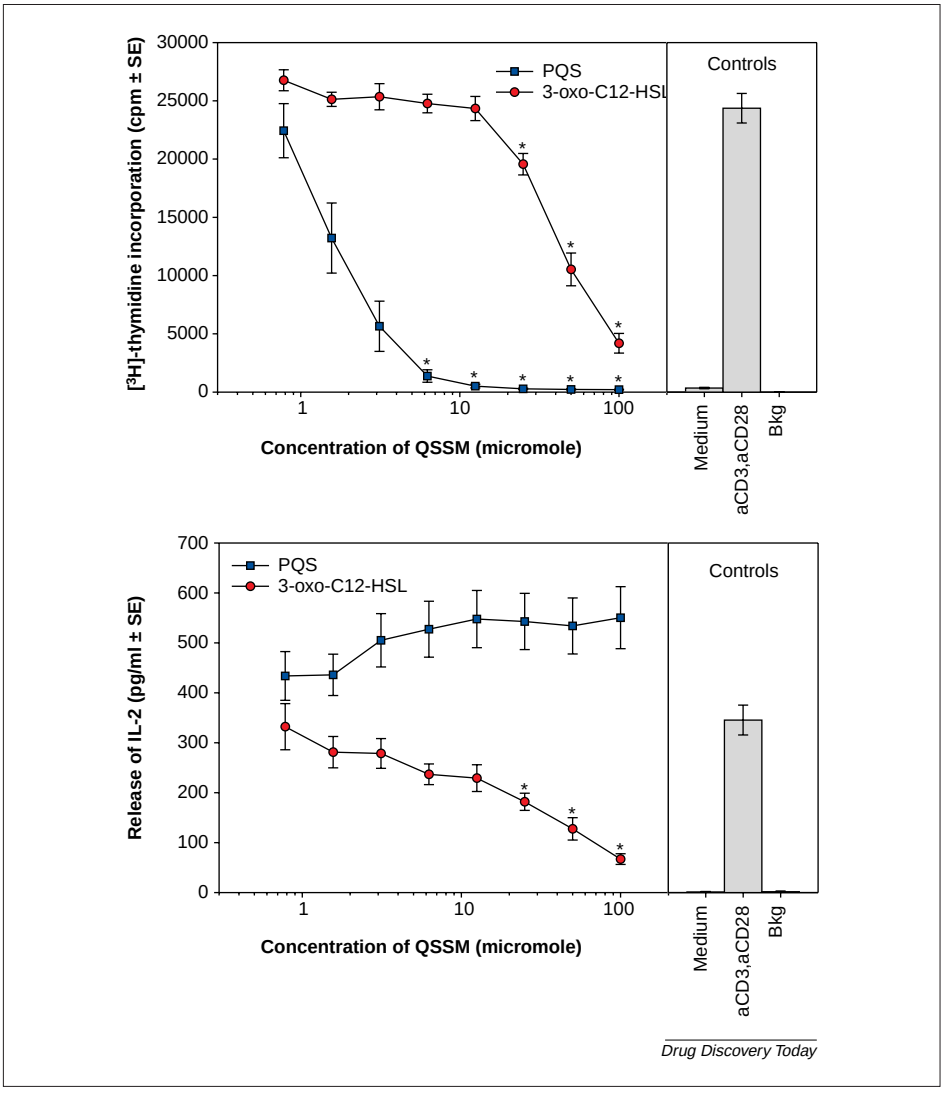

FIGURE 2

The effect of *Pseudomonas aeruginosa* quorum-sensing signalling molecules (QSSMs) on the proliferation of, and IL-2 secretion from, human peripheral bloodmononuclear cells activated using anti-CD3/anti-CD28 antibodies.

Evidence that this approach can produce chemical novelty

To date, we have identified:

- Two distinct chemical entities (abbreviated to OdDHL and PQS – Figure 1) from *Pseudomonas aeruginosa* [13], which suppress T-cell proliferation upstream and downstream of IL-2 secretion (c.f. cyclosporin and rapamycin; Figure 2). These are bacterial signal molecules associated with a process termed 'quorum sensing'. There is evidence that one of these compounds inhibits the JAK/STAT signalling system [27]. Inhibitors of JAK/STATs and kinases represent a possible new cluster of drugs for the treatment of immunological diseases such as asthma [28,29].
- Two low molecular mass, non-proteinaceous immune suppressive factors from the nematode parasites *Necator americanus* and *Heligmosomoides polygyrus*, which target T-cells, and induce apoptosis only in activated T-cells [22,23]. The source organisms suppress lymphocyte migration into an inflamed gut, act systemically from the gut, and could moderate the appearance of asthma, driven by dust mite allergens, in communities where the parasite infection is endemic [24]. Although the exact chemical nature of these immune

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suppressive agents has yet to be discovered, the medical community has been sufficiently impressed with the potential of nematode-induced immune modulation to initiate clinical trials using live infections with human hookworms to moderate allergy (City Hospital, Nottingham, UK). Another related nematode parasite, *Trichuris suis*, is currently being used in our attempt to alleviate inflammatory bowel diseases [30,25].

Obviously, this early success might not translate into the 'numbers game' preferred by some. For example, Xenova screened 100,000 microbial extracts for inhibitors of CD28 signal transduction [31]. However, the numbers generated by screening judiciously selected organisms could translate more quickly, and with less attrition, to a therapeutic end product.

Conclusion

As the pharmaceutical industry gears itself to mine the metabolomes of several 'traditional' organisms for new therapeutic leads, it is suggested that the industry looks closer to home, within the human body itself and at its parasitic load. Parasitic organisms have evolved over generations of co-existence with the human host to produce molecules to suppress the immune system in a subtle manner, to promote their own survival without causing excessive damage to, and ensuring survival of, the host on which it is so dependent. This is where new therapeutics lie for the treatment of autoimmune diseases, allergy and the promotion of graft survival. The methodology for compound isolation exists [32]. Is the pharmaceutical industry audacious enough to look?

Acknowledgement

Professor Rick Titball (DstI – Porton Down) contributed significantly to the compilation of the organism list in Table 2. Finally, a big thank you to my father.

References

1 Borel, J.F. *et al.* (1976) Biological effects of cyclosporin A: A new antilymphocyte agent. *Agents Action* 6, 468–475
2 Schreiber, S.L. and Crabtree, G.R. (1992) The mechanism of action of cyclosporin A and FK506. *Immunol. Today* 13, 136–142
3 Sehgal, S.N. (2003) Sirolimus: Its discovery, biological properties, and mechanism of action. *Transplant. Proc.* 35, 7S–14S
4 Harding, M.W. *et al.* (1989) A receptor for the immunosuppressant FK506 is a *cis-trans* peptidyl-prolyl isomerase. *Nature* 341, 758–760
5 Fretz, H. *et al.* (1991) Rapamycin and FK506 binding proteins (Immunophilins): Communications to the Editor. *J. Am. Chem. Soc.* 113, 1409–1411
6 Allison, A.C. (2000) Immunosuppressive drugs: the first 50 years and a glance forward. *Immunopharmacology* 47, 63–83
7 Perić-Concha, N. and Long, P.F. (2003) Mining the microbial metabolome: a new frontier for natural product lead discovery. *Drug Discov. Today* 8, 1078–1084
8 Haefner, B. (2003) Drugs from the deep: marine natural products as drug candidates. *Drug Discov. Today* 8, 536–544
9 Simmonds, M.S.J. (2003) Novel drugs from botanical sources. *Drug Discov. Today* 8, 721–722
10 Harvey, A. (2000) Strategies for discovering drugs from previously unexplored natural products. *Drug Discov. Today* 5, 294–300
11 Akbari, O. *et al.* (2003) Role of regulatory T cells in allergy and asthma. *Curr. Opin. Immunol.* 15, 627–633
12 Telford, G. *et al.* (1998) The *Pseudomonas aeruginosa* quorum-sensing signal molecule *N*-(3-Oxododecanoyl)-L-homoserine lactone has immunomodulatory activity. *Infect. Immun.* 66, 36–42
13 Hooi, D.S.W. *et al.* (2004) Differential immune modulatory activity of *Pseudomonas aeruginosa* quorum sensing signal molecules (QSSM). *Infect. Immun.* 72, 6463–6470
14 Zhou, L. *et al.* (2004) *Yersinia* YopJ inhibits pro-inflammatory molecule expression in human bronchial epithelial cells. *Respir. Physiol. Neurobiol.* 140, 89–97
15 Pujol, C. and Bliska, J.B. Turning *Yersinia* pathogenesis outside in: subversion of macrophage function by intracellular yersiniae. *Clin. Immunol.* (in press)
16 Guidi-Rontani, C. (2002) The alveolar macrophage: the Trojan horse of *Bacillus anthracis*. *Trends Microbiol.* 10, 405–409
17 Letesson, J-J. *et al.* (2002) Fun stories about *Brucella*: the "furtive nasty bug". *Vet. Microbiol.* 90, 317–328
18 White, N.J. (2003) Melioidosis. *Lancet* 361, 1715–1722
19 Venturi, V. *et al.* (2004) Quorum sensing in the *Burkholderia cepacia* complex. *Res. Microbiol.* 155, 238–244
20 Hasan, Z. *et al.* (2003) The effect of mycobacterial virulence and viability on MAP kinase signalling and TNFα production by human monocytes. *Tuberculosis (Edinb.)* 83, 299–309
21 Mansour, M.K. and Levitz, S.M. (2002) Interactions of fungi with phagocytes. *Curr. Opin. Microbiol.* 5, 359–365
22 Telford, G. *et al.* (1998) *Heligmosomoides polygyrus* immunomodulatory factor (IMF), targets T-lymphocytes. *Parasite Immunol.* 20, 601–611
23 Pritchard, D.I. and Brown, A. (2001) Is *Necator americanus* approaching a mutualistic symbiotic relationship with humans? *Trends Parasitol.* 17, 159–207
24 Scrivener, S. *et al.* (2001) Independent effects of intestinal parasite infection and domestic allergen exposure on the risk of wheeze in Ethiopia. *Lancet* 358, 1493–1499
25 Elliott, D.E. *et al.* (2000) Does the failure to acquire helminthic parasites predispose to Crohn's disease? *FASEB J.* 14, 1848–1855
26 Lavelle, E. *et al.* (2004) Molecules of infectious agents as immunomodulatory drugs. *Curr. Top. Med. Chem.* 4, 499–508
27 Li, L. *et al.* (2004) Bacterial *N*-acylhomoserine lactone-induced apoptosis in breast carcinoma cells correlated with down-modulation of STAT3. *Oncogene* 23, 4894–4902
28 Luo, C. and Laaja, P. (2004) Inhibitors of JAKs/STATs and the kinases: a possible new cluster of drugs. *Drug Discov. Today* 9, 268–275
29 Wong, W.S.F. and Leong, K.P. (2004) Tyrosine kinase inhibitors: a new approach for asthma. *Biochim. Biophys. Acta* 1697, 53–69
30 Falcone, F.H. and Pritchard, D.I. Parasite role reversal: worms on trial. *Trends Parasitol.* (in press)
31 Sadeghi, R. *et al.* (2001) Differential regulation of CD3- and CD28-induced IL-2 and IFN-γ production by a novel tyrosine kinase inhibitor XR774 from Cladosporium cf. cladosporioides. *Int. Immunopharmacol.* 1, 33–48
32 Abel, U. *et al.* (2002) Modern methods to produce natural-product libraries. *Curr. Opin. Chem. Biol.* 6, 453–458

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