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The 'hygiene hypothesis' for autoimmune and allergic diseases: an update

H Okada, C Kuhn, H Feillet, and J-F Bach

INSERM U1013, Necker-Enfants Malades Hospital, Paris, France
J.-F. Bach, INSERM U1013, Hôpital Necker-Enfants Malades, 161 rue de Sèvres 75015 Paris, France. E-mail: jean-francois.bach/at/academie-sciences.fr

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ABSTRACT

According to the 'hygiene hypothesis', the decreasing incidence of infections in western countries and more recently in developing countries is at the origin of the increasing incidence of both autoimmune and allergic diseases. The hygiene hypothesis is based upon epidemiological data, particularly migration studies, showing that subjects migrating from a low-incidence to a high-incidence country acquire the immune disorders with a high incidence at the first generation. However, these data and others showing a correlation between high disease incidence and high socio-economic level do not prove a causal link between infections and immune disorders. Proof of principle of the hygiene hypothesis is brought by animal models and to a lesser degree by intervention trials in humans. Underlying mechanisms are multiple and complex. They include decreased consumption of homeostatic factors and immunoregulation, involving various regulatory T cell subsets and Toll-like receptor stimulation. These mechanisms could originate, to some extent, from changes in microbiota caused by changes in lifestyle, particularly in inflammatory bowel diseases. Taken together, these data open new therapeutic perspectives in the prevention of autoimmune and allergic diseases.

Keywords: allergy, autoimmunity, regulatory T cells

INTRODUCTION

Changes of lifestyle in industrialized countries have led to a decrease of the infectious burden and are associated with the rise of allergic and autoimmune diseases, according to the 'hygiene hypothesis'. The hypothesis was first proposed by Strachan, who observed an inverse correlation between hay fever and the number of older siblings when following more than 17 000 British children born in 1958 [1]. The original contribution of our group to the field was to propose for the first time that it was possible to extend the hypothesis from the field of allergy, where it was formulated, to that of autoimmune diseases such as type 1 diabetes (T1D) or multiple sclerosis (MS) [2]. The leading idea is that some infectious agents – notably those that co-evolved with us – are able to protect against a large spectrum of immune-related disorders. This review summarizes in a critical fashion recent epidemiological and immunological data as well as clinical studies that corroborate the hygiene hypothesis.

The strongest evidence for a causal relationship between the decline of infections and the increase in immunological disorders originates from animal models and a number of promising clinical studies, suggesting the beneficial effect of infectious agents or their composites on immunological diseases.

In this review, we shall attempt to evaluate the arguments in favour of the hygiene hypothesis with particular interest on the underlying mechanisms.

EVOLVING EPIDEMIOLOGY OF ALLERGIC AND AUTOIMMUNE DISEASES

The rising incidence of atopic and autoimmune diseases

In 1998, about one in five children in industrialized countries suffered from allergic diseases such as asthma, allergic rhinitis or atopic dermatitis [3]. This proportion has tended to increase over the last 10 years, asthma becoming an 'epidemic' phenomenon [4]. The increasing prevalence of asthma is important in developed countries (more than 15% in United Kingdom, New Zealand and Australia) but also in developing countries, as illustrated by a prevalence greater than 10% in Peru, Costa Rica and Brazil. In Africa, South Africa is the country with the highest incidence of asthma (8%) [5]. Unfortunately, data from most other African countries are unavailable [6]. The prevalence of atopic dermatitis has doubled or tripled in industrialized countries during the past three decades, affecting 15–30% of children and 2–10% of adults [7]. In parallel, there is also an increase in the prevalence of autoimmune diseases such as T1D, which now occurs earlier in life than in the past, becoming a serious public health problem in some European countries, especially Finland, where an increasing number of cases in children of 0–4 years of age has been reported [8]. The incidence of inflammatory bowel diseases (IBD), such as Crohn's disease or ulcerative colitis [2] and primary biliary cirrhosis [9] is also rising. Part of the increased incidence of these diseases may be attributed to better diagnosis or improved access to medical facilities in economically developed countries. However, this cannot explain the marked increase in immunological disorder prevalence that has occurred over such a short period of time in those countries, particularly for diseases which can be diagnosed easily, such as T1D or MS [10–12].

The decreasing incidence of infectious diseases

Public health measures were taken after the industrial revolution by western countries to limit the spread of infections. These measures comprised decontamination of the water supply, pasteurization and sterilization of milk and other food products, respect of the cold chain procedure, vaccination against common childhood infections and the wide use of antibiotics. The decline is particularly clear for hepatitis A (HAV), childhood diarrhoea and perhaps even more spectacular for parasitic diseases such as filariasis, onchocercosis, schistosomiasis or other soil-transmitted helminthiasis [13]. In countries where good health standards do not exist, people are chronically infected by those various pathogens. In those countries, the prevalence of allergic diseases remains low. Interestingly, several countries that have eradicated those common infections see the emergence of allergic and autoimmune diseases.

Uneven distribution

The geographical distribution of allergic and autoimmune diseases is a mirror image of the geographical distribution of various infectious diseases, including HAV, gastrointestinal infections and parasitic infections. There is an overall North–South gradient for immune disorders in North America [14], Europe [2] and also in China [15] with intriguing exceptions such as asthma in South America or T1D and MS in Sardinia. There is also a West–East gradient in Europe: the incidence of T1D in Bulgaria or Romania is lower compared to western Europe, but is increasing fast [16]. This gradient cannot be fully explained by genetic differences. Indeed, the incidence of diabetes is sixfold higher in Finland compared to the adjacent Karelian republic of Russia, although the genetic background is the same [17].

Additionally, migration studies have shown that offspring of immigrants coming from a country with a low incidence acquire the same incidence as the host country, as rapidly as the first generation for T1D [18] and MS [19,20]. This is well illustrated by the increasing frequency of diabetes in families of immigrants from Pakistan to the United Kingdom [21] or the increasing risk of MS in Asian immigrants moving to the United States [22]. The prevalence of systemic lupus erythematosus (SLE) is also much higher in African Americans compared to West Africans [23].

These data do not exclude the importance of genetic factors for those immunological disorders, as assessed by the high concordance of asthma, T1D or IBD in monozygotic twins: for example, the concordance rate for atopic dermatitis among monozygotic twins is high (77%) compared to dizygotic twins (15%) [7]. The difference in some genetic factors according to ethnicity [human leucocyte antigen (HLA) gene difference between Caucasian and Asian, for example] is well documented, but probably plays a minor role in geographical distribution in view of migrant data.

Search for risk factors at the origin of the increase of immunological disorders

Several factors, such as socio-economic indices, may explain the difference in the prevalence of immunological disorders according to time and geographical distribution. In fact, there is a positive correlation between gross national product and incidence of asthma, T1D and MS in Europe [2]. This is true at the country level, but also at that of smaller regions, such as Northern Ireland, where the low incidence of T1D is correlated with low average socio-economic level, as evaluated by conventional indices [24]. Similar results have been obtained in the province of Manitoba in Canada for Crohn's disease [25]. This correlation has even been demonstrated at the individual level for atopic dermatitis, as family income is correlated directly with the incidence of the disease [26]. However, this does not pinpoint which factor within the socio-economic indices is directly responsible for the immunological disorder. Several epidemiological studies have indicated a positive correlation between sanitary conditions and T1D [24] or MS [27], suggesting a possible role of infections. Other factors are often incriminated, such as air pollution for asthma [28], but their role has not been demonstrated convincingly. For example, it has been shown that in East Germany before the fall of the Berlin Wall, where the air pollution was greater, the incidence of asthma was lower than in West Germany [29].

Vitamin D production is linked to sun exposure, and has been shown recently to have immunomodulatory effects [30]. However, this does not explain the West–East gradient of T1D in Europe, or the huge difference between Finland and its neighbouring Karelian region, where people have the same sun exposure level [31].

Epidemiological data indicating a direct link between the decreasing level of infectious burden and the rising incidence of immunological disorders

Several epidemiological studies have investigated the protective effect of infectious agents in allergic and autoimmune diseases. The presence of one or more older siblings protects against development of hay fever and asthma [1], of MS [32] and also of T1D [33], as does attendance at day care during the first 6 months of life in the case of atopic dermatitis and asthma [34].

Interestingly, exposure to farming and cowsheds early in life prevents atopic diseases, especially if the mother is exposed during pregnancy [35,36]. It has also been shown that prolonged exposure to high levels of endotoxin during the first year of life protects from asthma and atopy [37]. However, these data have been contradicted by other studies showing an increased prevalence of asthma correlated with higher levels of endotoxins in urban housing [38,39]. The level of endotoxins is higher in farms as compared to cities, and subjects are in contact with a greater variety of microbial compounds in farms,

which could explain this discrepancy.

Do helminth parasites protect against atopy? Epidemiological data of cross-sectional studies revealed that Schistosoma infections have a strong protective effect against atopy, as reviewed recently [40]. Hookworms such as *Necator americanus* also seem to protect from asthma. In contrast, *Ascaris lumbricoides* and *Trichuris trichiura* have no significant effect on disease. Parasitic infections have been almost eradicated in European countries since the Second World War, concomitant with the increase of atopy and allergy. This trend can explain part of the epidemiological difference between Europe and Africa, but cannot explain readily the intra-European North–South gradient.

PROOF OF PRINCIPLE OF THE CAUSAL RELATIONSHIP BETWEEN DECLINE OF INFECTIOUS DISEASES AND INCREASE OF IMMUNOLOGICAL DISORDERS

We have seen that there is a strong correlation between changes in lifestyle and modifications of the incidence of allergic or autoimmune diseases, but this does not prove a causal relationship between these two observations. This is a crucial question, as many factors unrelated to infections are a consequence of lifestyle, such as food habits, quality of medical care or dinner time gradient from North to South Europe. The answer to this question comes from animal models of autoimmune and allergic diseases and, to a lesser degree, from clinical intervention studies.

Animal models

The incidence of spontaneous T1D is directly correlated with the sanitary conditions of the animal facilities, for both the non-obese diabetic (NOD) mouse [2] and the bio-breeding diabetes-prone (BB-DP) rat [41]: the lower the infectious burden, the higher the disease incidence. Diabetes has a very low incidence and may even be absent in NOD mice bred in ‘conventional’ facilities, whereas the incidence is close to 100% in female mice bred in specific pathogen-free (SPF) conditions. Conversely, infection of NOD mice with a wide variety of bacteria, virus and parasites protects completely (‘clean’ NOD mice) from diabetes [2]. Similarly, mycobacteria (e.g. complete Freund's adjuvant) prevent induction of experimental autoimmune encephalomyelitis [42] and ovalbumin-induced allergic asthma [43]. Data obtained in our laboratory show that living pathogens are not required, as bacterial extracts are sufficient to afford protection [44].

Increased atopy after anti-parasitic treatments

It has been shown that helminth eradication increases atopic skin sensitization in Venezuela [45], in Gabon [46] and in Vietnam [40]. However, in a small study of 89 Venezuelan adults and children with asthma there was a clinical improvement, and specific immunoglobulin E (IgE) levels decreased after anti-helminthic treatment [47], while a similar deworming treatment showed no effect in Ecuador [48]. It is difficult to explain these contradictory data, which may relate to the complexity of asthma pathophysiology. In the same vein, one might also mention the increased atopy observed after vaccination with *Streptococcus pneumoniae* in South Africa [49].

Prevention of allergic and autoimmune diseases by infections

In a prospective study in Argentina, 12 patients with MS with high peripheral blood eosinophilia were followed. These patients presented parasitic infections and showed a lower number of MS exacerbations, increased interleukin (IL)-10 and transforming growth factor (TGF)-β secretion by peripheral blood mononuclear cells (PBMC) [50].

Similarly, deliberate administration of ova from the swine-derived parasite *Trichuris suis*, every 3 weeks

for 6 months to 29 patients with active Crohn's disease, improved symptoms in 21 of 29 patients (72%) with no adverse events [51]. *T. suis* ova were also given to patients with active ulcerative colitis, with significant improvement (43% improvement *versus* 17% for placebo) [52].

Another helminth, *Necator americanus*, has also been used in Crohn's disease, patients being inoculated subcutaneously with infective larvae. There was a slight improvement of symptoms, but the disease reactivated when immunosuppressive drugs were reduced [53].

Probiotics

Probiotics are non-pathogenic microorganisms that are assumed to exert a positive influence on host health and physiology [54]. Encouraging results were first shown in a double-blind randomized placebo-controlled trial in Finland, where *Lactobacillus GG* taken daily by expectant mothers for 2–4 weeks before delivery and postnatally for 6 months could decrease significantly the incidence of atopic dermatitis [55]. Perinatal protection lasted up to 7 years [56]. Another trial showed improvement of atopic dermatitis using other strains of probiotics [57]. However, a German group using the same protocol did not find any protective effect after 2 years [58]. Additionally, a recent study of 445 pregnant women in Finland who were treated with the same protocol as the initial Finnish study, but with freeze-dried *Lactobacillus GG*, failed to show any significant effect on eczema, allergic rhinitis or asthma 5 years after treatment. The only difference observed was a decreased IgE-associated allergic disease in caesarean-delivered children [59].

In T1D, only experimental data are available. The protective effect of a probiotic [60] and a bacterial extract [44] was reported on the onset of diabetes in NOD mice. A pilot study in humans, the PRODIA study (probiotics for the prevention of beta cell autoimmunity in children at genetic risk of type 1 diabetes), was begun in 2003 in Finland in children carrying genes associated with disease predisposition [61].

The case of probiotics in IBD is more complex because of the possible local anti-inflammatory effect, which could explain the relief of symptoms without changes in disease progression, as implicated in the hygiene hypothesis. Following a number of uncontrolled studies in a small cohort of 14 paediatric patients with newly diagnosed ulcerative colitis, probiotic treatment induced a significant rate of remission compared to the control group (93% *versus* 36%) and a lower relapse rate [62].

In brief, there are data suggesting that probiotics may have a favourable role in immune disorders, but the case is far from proven and requires further investigation. Additionally, although side effects are very low they might not be non-existent, as shown in a set of patients with acute pancreatitis [63]. Thus, probiotics should not be considered as totally harmless, particularly in the immunodeficient host, and more safety studies are needed. As mentioned by Sharp *et al.*, ‘probiotics may have unpredictable behaviour like all microorganisms, such as unanticipated gene expression in non-native host environment, or acquired mutations occurring spontaneously via bacterial DNA-transfer mechanisms’.

Is there a role for microbiota changes in the hygiene hypothesis?

The human gut is the natural niche for more than 10^{14} bacteria of more than 1000 different species [64]. Immediately after birth, the human gut is colonized with different strains of bacteria. This commensal microbiota is important in shaping the immune system, for other basic physiological functions [65] as well as for the integrity of the intestinal barrier [66]. Interestingly, the intestinal flora was different in a small group of allergic Estonian and Swedish children compared to the control group, with a higher count of aerobic bacteria such as coliforms and *Staphylocccus aureus* and a decreased proportion of

Lactobacilli, or anaerobes such as *Bifidobacterium* or *Bacteroides*[67,68]. However, this difference was not seen in a larger birth cohort study comparing three European baby populations [69]. Additionally, this study showed a slower acquisition of typical faecal bacteria such as *Escherichia coli*, especially in children delivered by caesarian section or children without siblings. It should be noted that all these studies were based on the analysis of culturable bacteria, and only atopic dermatitis and skin prick test were evaluated.

In autoimmune diseases the microbiota also seems to modulate the immune response. In NOD mice deficient for the myeloid differentiation primary response gene 88 (MyD88) signalling molecule it has been shown that microbiota protect mice from diabetes via a MyD88-independent pathway [70]. Using the metagenomic approach, it has been demonstrated that the biodiversity of the faecal microbiota of patients with Crohn's disease is diminished, especially for the Firmicutes phylum [71,72]. *Faecalibacterium prautsnitzii* is one of the Firmicutes that was particularly depleted, and it has been shown that this deficient commensal bacterium could improve IBD in a murine model of the disease [73]. This protective effect was also obtained with the supernatant of *F. prautsnitzii* culture, demonstrating the importance of one of the secreted molecules for its anti-inflammatory effect. Another bacterium, *Bacteroides fragilis*, has also been shown to protect animals from experimental colitis, and this protective effect was linked to a single microbial molecule, polysaccharide A [74]. As mentioned above, with regard to IBD these data must be interpreted with caution before extrapolating to other autoimmune disorders where the disease site is extra-intestinal. First, the respective anti-inflammatory and immunomodulatory effects of protective bacteria remain to be determined. Secondly, this protective effect should be discussed in the context of disease-promoting bacteria such as *Helicobacter hepaticus*.

In brief, there is an increasing amount of data showing that microbiota changes could contribute to the modulation of immune disorders but evidence is still slim, except in IBD. It is to be hoped that studies which provide a fair description of the molecular changes following intestinal infections will help in analysing the question further. The recent report by Fumagalli *et al*. is a good illustration of this new approach [75].

MECHANISMS OF THE HYGIENE HYPOTHESIS

When considering the multitude of infectious agents that can induce protection from various immunological disorders, it is not surprising that more than one single mechanism has been found.

T helper type 1 (Th1)–Th2 deviation

Th1–Th2 deviation was the first major candidate mechanism for explaining the protective influence of infectious agents from immunological disorders. Th1 T cells produce inflammatory cytokines such as IL-2, interferon (IFN)-γ and tumour necrosis factor (TNF)-α that are operational in cell-mediated immunity (including autoimmune diabetes). In contrast, Th2 T cells that produce IL-4, IL-5, IL-6 and IL-13 contribute to IgE production and allergic responses. Given the reciprocal down-regulation of Th1 and Th2 cells, some authors suggested initially that in developed countries the lack of microbial burden in early childhood, which normally favours a strong Th1-biased immunity, redirects the immune response towards a Th2 phenotype and therefore predisposes the host to allergic disorders. The problem with such an explanation is that autoimmune diseases, which in most cases are Th1 cell-mediated, are protected by infections leading to a Th1 response and that atopy may be protected, as seen above, by parasites which induce a Th2 response. These observations fit with the concept of a common mechanism underlying infection-mediated protection against allergy and autoimmunity. Several hypotheses may explain these common mechanisms.

Antigenic competition /homeostasis

It has been known for several decades that two immune responses elicited by distinct antigens occurring simultaneously tend to inhibit each other. Numerous mechanisms were evoked to explain antigenic competition that might be pertinent to the hygiene hypothesis. The development of strong immune responses against antigens from infectious agents could inhibit responses to 'weak' antigens such as autoantigens and allergens. Among the mechanisms that explain antigenic competition, attention has been drawn recently to lymphocyte competition for cytokines, recognition for major histocompatibility complex (MHC)/self-peptide complexes and growth factors necessary to the differentiation and proliferation of B and T cells during immune responses within the frame of lymphocyte homeostasis. Similarly to red blood cell mass, which is restored to normal levels after a haemorrhage with the help of erythropoietin, CD4 and CD8 T lymphocytes are restored to normal levels after a lymphopenia. Homeostatic factors that play an equivalent role to that of erythropoietin have not been elucidated completely; however, cytokines such as IL-2, IL-7, and IL-15 are known to play a crucial role. Regulatory T cells that we discuss below may also be implicated in the mechanism of antigenic competition.

Immunoregulation

Another mechanism involves regulatory T cells which can suppress immune responses distinct from reponses against the antigen in question, here antigens expressed by infectious agents (a phenomenon called bystander suppression). The problem is complicated by the multiplicity of regulatory lymphocytes involving diverse cytokines that mediate their differentiation or their regulatory effects. The role of $CD4^+CD25^+$forkhead box P3 ($FoxP3^+$) T cells has been suggested by transfer experiments performed in a murine parasite model [76]. The role of such cells is also suggested by the observation that $CD28^{-/-}$ NOD mice devoid of $CD4^+CD25^+$ $FoxP3^+$ regulatory T cells (T_{regs}) lose their sensitivity to the protective effect of bacterial extract (our unpublished data). It has also been reported that in cord blood from newborns of mothers exposed to farming, $CD25^+$FoxP3 cells were up-regulated [77]. This observation should be interpreted with caution because of the uncertain specificity of these markers in man.

Other data suggest a role for IL-10-producing B cells [78], natural killer (NK) T cells [79] and more generally cytokines such as IL-10 [80] and TGF-β[81] whatever the cell type producing these cytokines. More work is needed in experimental models to delineate further the involvement of regulatory mechanisms in the protective effects of the various infections relevant to the hygiene hypothesis. It might emerge that different mechanisms are operational according to the protective infection.

Non-antigenic ligands

All the mechanisms mentioned previously are based on the notion that the hygiene effect is due to the decrease of immunological responses elicited against infectious agents. A number of experiments indicate that infectious agents can promote protection from allergic diseases through mechanisms independent of their constitutive antigens, leading to stimulation of non-antigen specific receptors. This concept is well illustrated by the example of Toll-like receptors (TLRs). Knowing the capacity of TLRs to stimulate cytokine production and immune responses, it might be predicted that TLR stimulation by infectious ligands should trigger or exacerbate allergic and autoimmune responses. This has indeed been demonstrated in some experimental models [82,83].

Surprisingly, and paradoxically, it has also been observed that TLR stimulation could prevent the onset of spontaneous autoimmune diseases such as T1D in NOD mice, an observation made for TLR-2, -3, -4, -7 and -9 [84] (and our unpublished data). In this model, treatment with TLR agonists before disease onset prevents disease progression completely. The mechanisms underlying such protections are still ill defined, but could involve production of immunoregulatory cytokines and the induction of regulatory T

cells or NK T cells. Similar data have been observed in an ovalbumin-induced model of asthma [85].

Concerning HAV, it was shown initially that atopic diseases were less common in subjects that have been exposed to the virus [86]. It was difficult to say whether this association was due to a direct protective effect of HAV infection or explained only by the fact that HAV exposure is a matter of poor hygiene. Data obtained by Umetsu *et al*. have shown that HAV could influence T cells directly, notably Th2 cells that express the HAV receptor [87], a finding corroborated by the observation that atopy prevalence is associated with HAV receptor gene polymorphisms in anti-HAV antibody-positive subjects. In fact, recent data indicate that the HAV receptor, the TIM-1 protein (T cell, immunoglobulin domain and mucin domain), could play an important role in the severity of HAV and its putative effect on atopic diseases.

Gene–environment interactions

An interesting approach to identify mechanisms underlying allergic and autoimmune diseases consists in searching for associations between these diseases and polymorphisms of various genes, notably those coding for molecules involved in immune responses. It is interesting to note that such an association has been found for genes implicated in the control of infection. Among them, polymorphism in genes of the innate immune response such as *CD14*, *TLR2*, *TLR4*, *TLR6* or *TLR10*, and intracellular receptors such as NOD1 and NOD 2 [also known as caspase-recruitment domain (CARD)4 and CARD15, respectively], appears to be important [88,89]. Mouse studies have shown that these gene–environment interactions explain a proportion of the phenotypic variance. One of those genes is *CD14*, which is important in lipopolysaccharide (LPS)/TLR-4 signalling. Many association studies have highlighted the role of the CD14–159CT polymorphism and allergic inflammation [90].

THERAPEUTIC STRATEGIES

The notions presented above open new, interesting, therapeutic perspectives for the prevention of allergic and autoimmune diseases. Of course, contaminating children or adults at high risk of developing these diseases by infectious agents cannot be envisioned, at a time when medical progress has allowed the reduction of major infectious diseases. It should be mentioned, however, that even if we do not believe that this is not the best strategy for the future, some groups have used living parasites such as *T. suis* in the prevention of IBD, as mentioned above, or living Lactobacilli in the prevention of atopic dermatitis. These approaches present the obvious limitation of insufficient standardization, and hazards linked to unpredictable disease course in subjects presenting an unknown immunodeficiency by contamination with xenogeneic virus in the case of swine-derived parasites.

Conversely, the use of bacterial extracts, already shown to be efficacious in a number of experimental models and in the clinic, such as OM-85 in T1D, should be envisioned seriously [44]. These extracts, which represent the mixture of a wide spectrum of chemically ill-defined components, are also submitted to the criticism of poor standardization. On the other hand, they are a better representation of the various components of bacteria known for their protective effects. The same comments apply to parasitic extracts, shown to be effective in T1D [91]. In the long-term future, one would like to use chemically defined components of protective infectious agents, such as TLR agonists, polysaccharide A or the active substance secreted by *F. prautsnitzii*. In any event, the use of bacterial extracts or chemically defined products will be confronted with the double problem of the timing of administration (sufficiently early in the natural history of the disease), and of safety. Indeed, any side effects are unacceptable in young subjects who are apparently healthy and whose risk of developing the disease in question is not demonstrated absolutely.

DISCLOSURE

None of the authors has conflicts of interest to declare, or any relevant financial interest, in any company or institution that might benefit from this publication.

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