Published in final edited form as:
J Autoimmun. 2009 ; 33(3-4): 183–189. doi:10.1016/j.jaut.2009.09.001.

Environmental Triggers of Autoimmune Thyroiditis

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Abstract

Autoimmune thyroiditis is among the most prevalent of all the autoimmunities. Autoimmune thyroiditis is multifactorial with contributions from genetic and environmental factors. Much information has been published about the genetic predisposition to autoimmune thyroiditis both in experimental animals and humans. There is, in contrast, very little data on environmental agents that can serve as the trigger or autoimmunity in a genetically predisposed host. The best-established environmental factor is excess dietary iodine. Increased iodine consumption is strongly implicated as a trigger for thyroiditis, but only in genetically susceptible individuals. However, excess iodine is not the only environmental agent implicated as a trigger leading to autoimmune thyroiditis. There are a wide variety of other synthetic chemicals that affect the thyroid gland or have the ability to promote immune dysfunction in the host. These chemicals are released into the environment by design, such as in pesticides, or as a by-product of industry. Candidate pollutants include polyaromatic hydrocarbons, polybrominated biphenols, and polychlorinated biphenols, among others. Infections are also reputed to trigger autoimmunity and may act alone or in concert with environmental chemicals. We have utilized a unique animal model, the NOD.H2^{h4} mouse to explore the influence of iodine and other environmental factors on autoimmune thyroiditis.

Introduction

Autoimmune thyroiditis is among the most prevalent of all the autoimmunities, with an estimated number of new cases for 1996 to be over 40,000 [1]. The current incidence rate for thyroiditis/hypothyroidism in the United States is estimated at 21.8/100,000, over 90% of these cases are estimated to be women [1]. Autoimmune thyroiditis ranks third among the most frequent autoimmune diseases in the United States [1] and in adults is the most frequent cause of hypothyroidism [2].

Autoimmune thyroiditis, also known as Hashimoto's thyroiditis, is an organ-specific autoimmune disorder, characterized by infiltration of the thyroid gland by inflammatory cells, often followed by hypothyroidism due to destruction of the thyroid follicles and eventual fibrous replacement of the parenchymal tissue. Autoantibodies to thyroid-specific antigens also develop. The two primary antigens in autoimmune thyroiditis are thyroglobulin (Tg) and thyroperoxidase (TPO). Tg is a glycoprotein with a molecular weight of about 660 kDA that constitutes the storage form of thyroid hormones within the thyroid follicle. TPO is the enzyme located at the apical border of the thyroid cell that is responsible for iodinating Tg and producing the thyroid hormones. The clinical diagnosis of autoimmune thyroiditis depends on both

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physical and biochemical abnormalities as well as serological demonstration of autoantibodies to these major thyroid antigens [3].

Autoimmune disease is multifactorial in that a genetic predisposition combines with environmental risk factors to promote disease. Autoimmune thyroiditis is clearly such a multifactorial disease. Early evidence that thyroiditis has a hereditary component stems from studies of familial aggregation (reviewed in [4]. Our own studies of juveniles with autoimmune thyroiditis showed a definite genetic propensity for thyroid autoimmunity to run in families [5]. Further evidence for the genetic control of autoimmune thyroiditis stems from observations of twins. Monozygotic twins showed a higher concordance rate of disease than did dizygotic twins [6]. However, even with identical twins the concordance rate was only about 50%, emphasizing that other important factors such as the environment play a role in disease pathogenesis.

Considerable information has been published about the genetic predisposition to autoimmune thyroiditis in both experimental animals and humans. In contrast, only few reports have been published on environmental agents that can serve as the “trigger” of autoimmunity in a genetically predisposed host. Both infectious microorganisms and environmental chemicals have been implicated, based primarily on anecdotal evidence. The study of environmental agents requires the availability of a reproducible model of a genetically predisposed animal in which such putative agents can be tested.

The best-established environmental factor promoting the development of autoimmune thyroiditis is excess dietary iodine. Iodination of salt in the 1920s was introduced as a public health measure. The program was very successful and in the United States reduced the frequency of endemic goiter in school children in the Midwest from 26 – 70% to 1 – 4% [7]. This major public health victory of virtually eliminating endemic goiter in the United States, however, caused another set of entirely different problems. There is evidence that the incidence of autoimmune thyroiditis increased concomitantly with the increased iodine content in the diet [8]. The Mayo Clinic, for example, reported an increase in the number of diagnosed cases of chronic lymphocytic thyroiditis from 2/year in the 1930's to approximately 500 new cases per year in 1985 [9].

This increased iodine consumption is strongly implicated as a trigger for thyroiditis, but only in genetically susceptible individuals [10,11]. The thyroid gland in adults requires approximately 75 to 100 ug of iodine/day to maintain a steady state between uptake and secretion of hormones from the thyroid. Although the average daily requirement has been set at 70 ug (160–200 ug for adolescents) [12], the average daily iodine intake in the United States has been estimated at between 240 to 740 ug, depending on geographical location [7], indicating a continual iodine excess for most of the population of the United States. Sources of dietary iodine include food and food additives (kelp and seaweed, iodinated salt, iodine additives to bread/flour, preservatives, red coloring, therapeutics (amiodarone, vitamins, Lugol's solution, etc.), topical antiseptics, and contrast dyes, among others.

Renewed interest in the problem of excess iodine promoting autoimmune thyroiditis is reflected by several recent clinical research reports. A threefold increase in the prevalence of autoimmune thyroiditis among schoolchildren was noted once iodine deficiency was eliminated in an area of endemic goiter in northwestern Greece [13]. Concomitantly, iodine restriction in many patients with primary hypothyroidism restored normal thyroid function [14,15]. However, these reports were limited in scope to clinical and laboratory diagnostic findings. No mechanism for how iodine may promote or induce autoimmune thyroiditis was proposed. Work performed by our laboratory and by others has demonstrated that highly iodinated Tg is more immunogenic than poorly iodinated Tg. Our studies in humans show that

both antibody and *in vitro* T cell responses decreased to background levels when Tg lacked iodine. Tg re-iodination restored these responses [16–18]. Differing iodine content of human Tg can both create new epitopes and render others inaccessible, as recognized by monoclonal antibodies [16]. Clearly, excess iodine is an important factor in certain individuals that compromise thyroid function leading to autoimmune thyroiditis. However, excess iodine is not the only environmental agent implicated as a trigger leading to autoimmune thyroiditis.

In humans the thyroid gland can be compromised by dietary factors other than iodine. These may include naturally occurring goitrogens found in legumes and plants, and certain drugs such as amiodarone and lithium. Furthermore, there is a wide variety of synthetic chemicals that affect the thyroid gland or have the ability to promote immune dysfunction in the host. These chemicals are released into the environment by design, such as in pesticides, or as a by-product of industry. Candidate pollutants include polyaromatic hydrocarbons (PAH), polybrominated biphenols (PBBs), and polychlorinated biphenols (PCBs), among others. Infections are also reputed to trigger autoimmunity and may act alone or in concert with environmental chemicals. [19].

An inherent problem is how to test these compounds in a controlled situation. Furthermore, the effect on the thyroid may be different depending on the genetics. Studies in man are not always informative as Nice studies often do not differentiate between individuals with or without an autoimmune genetic predisposition. Subsequently, most of the basic work has been performed using animal models [20] [21–26]. We have utilized a unique animal model, the NOD.H2^{h4} mouse to explore the influence of iodine and other environmental factors on autoimmune thyroiditis.

NOD.H2^{h4} mice and iodine

Investigators at Merck Laboratories in connection with their diabetes genetics program originally developed this animal model by crossing the non-obese diabetic (NOD) mouse with the B10.A(4R) mouse strain, and extensively backcrossing to the NOD. This new mouse strain was designated as NOD.H2^{h4}. The NOD.H2^{h4} has a MHC II background that is permissive for thyroiditis, the IA^{k} [27]. None of the mice developed diabetes. However, a high proportion showed evidence of thyroiditis in older animals (50% in NOD.H2^{h4} vs 5% in the NOD strain) not found in either of the parental strains. Furthermore, the incidence of thyroiditis in NOD.H2^{h4} rose to 90% when excess iodine was added to the drinking water [28].

In this NOD.H2^{h4} mouse model, we validated that the thyroiditis is autoimmune in origin [29]. We showed that there is a mononuclear infiltration in the thyroid gland (Figure 1) with a correlation between the dose of iodine and the severity of disease [30], shown in Figure 2. We further showed that disease could be transferred with spleen cells from NOD.H2^{h4} mice fed excess iodine in their drinking water into young, non-diseased animals ((Mann-Whitney rank sum test $p<.005$), results shown in Figure 3.

The NOD.H2^{h4} mouse is a spontaneous model of autoimmune thyroiditis that closely resembles human disease. The histology is similar to that of autoimmune thyroiditis in humans, and is characterized by chronic infiltration of mononuclear cells, including CD4, CD8, B cells, macrophages, and dendritic cells [31–35]. Further, the severity of disease correlates with antibody titers to Tg [32–34]. While excess iodine is not necessary for induction of thyroiditis, the ingestion of excess iodine works to exacerbate autoimmune thyroiditis in this genetically predisposed population.

Role of iodine

One of the striking observations we made in our ongoing studies of the NOD.H2^{h4} mouse is the constitutive expression of intracellular adhesion molecule-1 adhesion (ICAM-1) on thyrocytes [36]. Bonita et al. found that this expression of ICAM-1 was upregulated upon excess iodine ingestion, both in areas of cellular infiltration but also areas of the thyroid gland full killer cells without areas of cellular infiltration [36]. This suggests that iodine by itself could foster increased expression of ICAM-1. Subsequent work indeed suggested that iodine by itself increased ICAM-1 expression in the thyrocyte [37]. This led us to study the effect of iodide on the thyrocyte itself. Therefore, one mechanism to explain the accelerated mononuclear cell infiltration into the thyroid gland after iodine supplementation involves the local upregulation of ICAM-1. Subsequently, we learned that in the NOD.H2^{h4} mouse increased iodine consumption leads to an elevated reactive oxygen species (ROS) expression in the thyroid, which may be responsible for the elevated ICAM-1 expression in the thyrocyte [38]. Clearly, excess iodine intake has many roles, one is to increase thyroglobulin immunogenicity while another is to increase adhesion molecules on the thyrocyte itself.

The ICAM-1 promotor has multiple transcription binding sites with at least three different transcriptional initiation sites[39]. Multiple stimuli promote ICAM-1 expression besides cytokines including viruses, radiation, retinoic acid, and oxidants. The major intracellular signal transduction pathways involved in the regulation of ICAM-1 expression include protein kinase C (PKC), mitogen-activated protein (MAP) kinases, and the NF-kB signaling pathway [39]. The nuclear transcription factors important for the activation of ICAM-1 include AP-1, NF-kB, C/EBP, Ets, STAT and Sp1 [39]. Reports indicate that the induction of ICAM-1 transcription occurs rapidly, being detected as early as 30 minutes after stimulation [39]. This finding is in line with the observation that increased ICAM-1 expression is a very early step in the initiation of inflammation. Further, many other stimuli have been shown to facilitate ICAM-1 gene transcription [39].

How then might iodine upregulate ICAM-1 expression? Iodine is taken up by the thyrocyte, organified, and stored on the thyroglobulin molecule through the enzymatic reaction of thyroperoxidase. In doing so, there is the potential generation of ROS such as free radicals and an increase in the hydrogen peroxide generated during the enzymatic reaction. Both of these compounds are known to signal transcription of ICAM-1 [39].

Furthermore, other molecules such as interferon-gamma (IFN-g) act to upregulate ICAM-1 through the IFN-g responsive element (IRE) 100 bp upstream of the translation start site. IFN-g binds to its membrane receptor with subsequent phosphorylation of the Janus kinases, JAK-1 and 2 with subsequent phosphorylation of STAT1/3. The STATs then translocate to the nucleus, bind the IRE promotor sites leading to increased ICAM-1 expression. TNF-a, known to synergistically upregulate ICAM-1 along with IFN-g, apparently works through an independent pathway. This pathway utilizes the activation of NF-kB. Each pathway then works independently, resulting in synergy. In our studies we noted synergy in ICAM-1 expression on thyrocytes from NOD.H2^{h4} mice transgenic for thyroidal IFN-g [40]. This occurred only when the animals were treated with excess dietary iodine. This observation suggests that iodine and IFN-g also work by different pathways. This new knowledge of how iodine affects ICAM-1 expression may lead to determining new strategies in the quest for thyroiditis prevention in susceptible individuals.

We established that NOD-H2^{h4} mice have the genetic propensity for thyroiditis and at least one compound, iodine, promotes the onset of thyroiditis. However, as mentioned before, a few animals develop autoimmune thyroiditis even in the absence of excess iodine. This poses the question if there are other environmental agents that promote this disease. These NOD.H2^{h4}

mice are ideal for their use as a model sentinel animal to examine the influence of other environmental compounds on the development of autoimmune thyroiditis.

NOD.H2^{h4} and other environmental influences

SPF vs Conventional Housing

NOD.H2^{h4} mice were bred and maintained for most experimental procedures in pathogen free conditions (SPF) at the Johns Hopkins animal facility. A second colony of NODH2^{h4} mice were taken out of the SPF and bred and maintained in conventional housing at the same facility. Both colonies of mice (SPF and conventional) were maintained according to the guidelines from the Animal Care and Use Committee of the Johns Hopkins University. These untreated NOD.H2^{h4} mice were sacrificed at different ages and assessed for prevalence of infiltrating lymphocytes by H and E staining of paraffin embedded thyroid glands. The presence of lymphocytic infiltration as an indication of spontaneously developing disease was evaluated by two different examiners. Thyroid lesions were graded as previously described [41]. Mice in both housing facilities received similar care.

Results are presented in Figure 4. We observed that mice in conventional housing developed spontaneous thyroiditis at a low level as early as 20 weeks of age. By 40 weeks of age all mice in the conventional facility showed some degree of disease. The severity of thyroiditis appeared to increase as the animals aged as shown by regression analysis (R^2=0.44) (Figure 4A). In contrast, NOD.H2^{h4} mice maintained in SPF conditions did not develop disease before 30–39 weeks of age (Figure 4B). As the SPF mice aged the number of mice showing spontaneous thyroiditis increased slowly, but only after 39 weeks of age, with an almost 10 fold less regression than their counterparts in conventional housing(R^2 = 0.05). However, many mice showed no disease at all. These results show that NOD.H2^{h4} mice in conventional housing developed a greater frequency of spontaneous thyroiditis over those animals housed under SPF conditions. This is unlike the parental NOD strain wherein a protective environment leads to increased diabetes not decreased (reviewed in [42]). Our data indicate that prevailing intercurrent infection and micro-organisms or other elements in a non-protective environment appear to foster the induction of autoimmune thyroid disease in the NOD.H2^{h4} mouse.

Multipary

One condition in humans that associates with the onset of autoimmunity is pregnancy. The autoimmune thyroiditis that results from pregnancy is often postpartum and is frequently transient. However this happens primarily in a genetically predisposed population. For example, in women with previously-developed thyroid antibodies, the thyroiditis with resulting hypothyroidism often remains permanent [43]. Therefore, we examined the prevalence of thyroiditis in our NOD.H2^{h4} breeder colony compared to age matched non-parous female mice raised in either SPF or conventional housing (Figure 5).

Female NOD.H2^{h4} breeders raised in conventional housing developed significantly more thyroiditis than those breeders raised under SPF conditions ($p<0.01$, Fisher's Exact test); the frequency of thyroiditis in the conventionally-raised breeder group was also higher than the conventionally-raised non-breeder group, although the numbers were not sufficient to show significance. Female NOD.H2^{h4} mice raised in SPF conditions whether they were breeders or not showed similar frequencies of thyroiditis. These results imply that environmental factors when added to other unknown factors induced by pregnancy may promote thyroiditis in these mice.

Chemicals implicated in autoimmune thyroid disease

In a recent review by Brucker-Davis [44] over 90 synthetic chemicals were noted to show disruption of hormone balance or thyroid dysfunction. These chemicals arise from herbicides, insecticides, disinfectants, batteries, smoke, plasticizers, by products of combustion, petroleum, and flame retardants among others. Many of these compounds are widely distributed through the environment. However, only few environmental pollutants show evidence that they contribute to autoimmune thyroid disease. Most animals used in toxicology studies do not have a genetic predisposition for autoimmune disease. We know that in the iodine model of autoimmunity only animal strains with the correct genetic background develop an autoimmune response after excess iodine ingestion. A similar finding could also be true for testing of the other toxic compounds. Therefore, we used the NOD.H2^{h4} mouse as a model to test the influence of other compounds on the development of thyroiditis.

Polyaromatic Hydrocarbons (PAH)—Epidemiological work has implicated PAH in autoimmune disease in humans. Gaitan et al. have studied the effect of several organic pollutants on the thyroid function. The pollutants are organics produced from coal and found in air and water. In their study they found that these pollutants have potent anti-thyroid effects [45]. Two populations of school children from different geographical locations showed a high prevalence of goiter in equally iodine-sufficient areas [46]. However, only one group developed anti-thyroglobulin and anti-microsomal antigen autoantibodies. The investigators suggested that the PAH pollutants were triggers for the expression of autoimmune thyroiditis in a genetically predisposed population.

This group of chemicals found in smoke from combustion and as by products of petroleum has been shown to promote thyroiditis in rats. Several studies using the BUFF rat, a spontaneous model of thyroiditis, indicate that exposure by methylcholanthrene (MCA) or 7,12-methylbenz (a) anthracene, PAHs, increased the incidence of thyroiditis in younger animals [47,48]. In these studies thyroiditis was determined only by histology. Studies using MCA were continued by Silverman and Rose [49], who showed that the thyroiditis was associated with an autoantibody response to thyroglobulin and was virtually identical to the non-MCA form of the disease. Silverman and Rose pursued this model and found that there was a genetic susceptibility to the MCA-induced thyroiditis [50]. MCA serves as a prototype of a larger number of PAH to study the influence of these compounds on the promotion of thyroiditis in the genetically predisposed model, the NOD.H2^{h4} mouse. NOD.H2^{h4}mice were over five times more likely to develop thyroiditis when exposed to MCA than their untreated counterparts (Table 1).

Polyhalogenated Biphenyls (PBB)—Polyhalogenated biphenyls, such as polychlorinated biphenyls (PCB) or polybrominated biphenyls (PBB) are commonly used compounds with a wide variety of industrial applications. PBB are used as flame retardants, while PCB are used as lubricants, adhesives, inks and plasticizers. One study in humans found an unexpectedly high prevalence of primary hypothyroidism (11%) in workers from a factory producing PBB and PBB oxides [51]. The hypothyroidism was associated with an elevation of anti-microsomal thyroid antibodies and, less frequently, anti-thyroglobulin antibodies. The increase was highly unusual, especially since the patients were all men. The investigators compared the prevalence to a cohort from Whickham, England [52] and found a significant increase from that group with a $p<.001$, using the Z test. The investigators concluded that the increase was caused by the exposure to PBB, PBB oxide, or bromine. It may be that the bromine, by itself, may be causing the thyroid dysfunction since KBr or NaBr induces morphological changes in the thyroids of normal rats [53,54].

Using KBr as a surrogate for PBB, we had the capacity to show that bromine may exacerbate autoimmune thyroiditis in an animal with a genetic predisposition, the NOD.H2^{h4} mouse. Treatment with KBr increased the likelihood of thyroiditis in the NOD.H2^{h4} mice by a modest 1.5 times in the protected SPF environment (Table 1). However, the odds ratio increased to almost 4 times if the mice were treated with KBr in conventional housing, suggesting that even small "insults" along with exposure to environmental microorganisms may be additive leading to a higher incidence of thyroiditis.

Infection

Infections are reputed to contribute to the initiation of autoimmune disease. Little firm evidence implicates specific viruses or bacteria contributing to the pathogenesis of autoimmune thyroid disease [55]. It may well be that it is the bystander effect of activated T cells and the heightened immune response, in other words the adjuvant effect, that helps trigger autoimmunity. No single organism is responsible because one of multiple microorganisms could produce the same effect. NOD-H2h^4 mice were used to test the hypothesis that adjuvants alone are sufficient to trigger autoimmune thyroiditis in a genetically predisposed host. Bacterial lipopolysaccharide (LPS) is a bacterial product that is mitogenic for B cells in vitro but has also been shown to be a potent adjuvant for T cell-dependent antigens [56]. LPS treatment increased the likelihood of spontaneous thyroiditis over that present in untreated controls by almost 4 times (Table 1). While this was the only specific bacterial product tested, each time the treatment in SPF conditions was compared to the same treatment in conventional housing, the odds ratio of thyroiditis increased substantially. This indicates that potentially multiple, even non-life-threatening, normal infections may further act as triggers of thyroiditis in a genetically predisposed population.

Conclusions

There is strong evidence that environmental agents play a critical role in triggering autoimmune disease in genetically susceptible hosts. There is, however, little information about how such agents work. By using this well-defined mouse model, the NOD.H2^{h4} mouse, and a well-documented environmental trigger, iodine, we have determined at least one mechanism by which the fundamental issue of autoimmune thyroid disease pathogenesis can be established. Using the NOD.H2^{h4} mouse we have, in addition, established that this mouse model is appropriate for use in testing other environmental triggers of autoimmunity, such as chemicals and microorganisms. Further, we have shown that these triggers can be additive in nature. Even a mild-acting exposure, such as bromine administration, was potentiated by an additional exposure to microorganisms as present in conventional housing. While we have not yet identified specific organisms that promote the development of thyroiditis, we know that this phenomenon exists. Each time an environmental exposure was tested in both SPF and conventionally housed NOD.H2^{h4} mice, those animals exposed to the non-protected environment of conventional housing had more than a doubling of the likelihood of developing thyroiditis. This interaction between genes and environment may also very well be happening in the human population. However in humans, because of genetic heterogeneity and the diversity of environments and chemicals to which we are all exposed are so different, firmly establishing a causal relationship between environmental triggers and increased development of thyroiditis may be difficult and challenging. The importance of this unique animal model, the NOD.H2^{h4} mouse, in investigating, assessing, and delineating the contributions of the environmental factors acting as triggers or active contributors to the pathogenesis of autoimmune thyroid disease is of great value to medical and translational research. It is fitting that this issue be devoted to the contributions of Noel Rose and that this paper be focused on thyroiditis. The contributions of Noel Rose to thyroiditis are legion and we note that it is a subject which is continually important not only specifically for the dissection of human

autoimmune thyroiditis, but also generically important for autoimmunity and in that respect we cite recent literature from this journal which focuses on this very issue [57–65]. Lastly, we note that this is an issue as part of the Journal of Autoimmunity series devoted to major figures in the field of autoimmunity and immunology and certainly Noel Rose [66–70] fits very prominently for several decades in this unique group [71–74].

Acknowledgments

This work was supported in part by NIH grant R21 ES10285

I wish to thank Noel Rose for his continual support throughout the many years that I have had the privilege to work with him.

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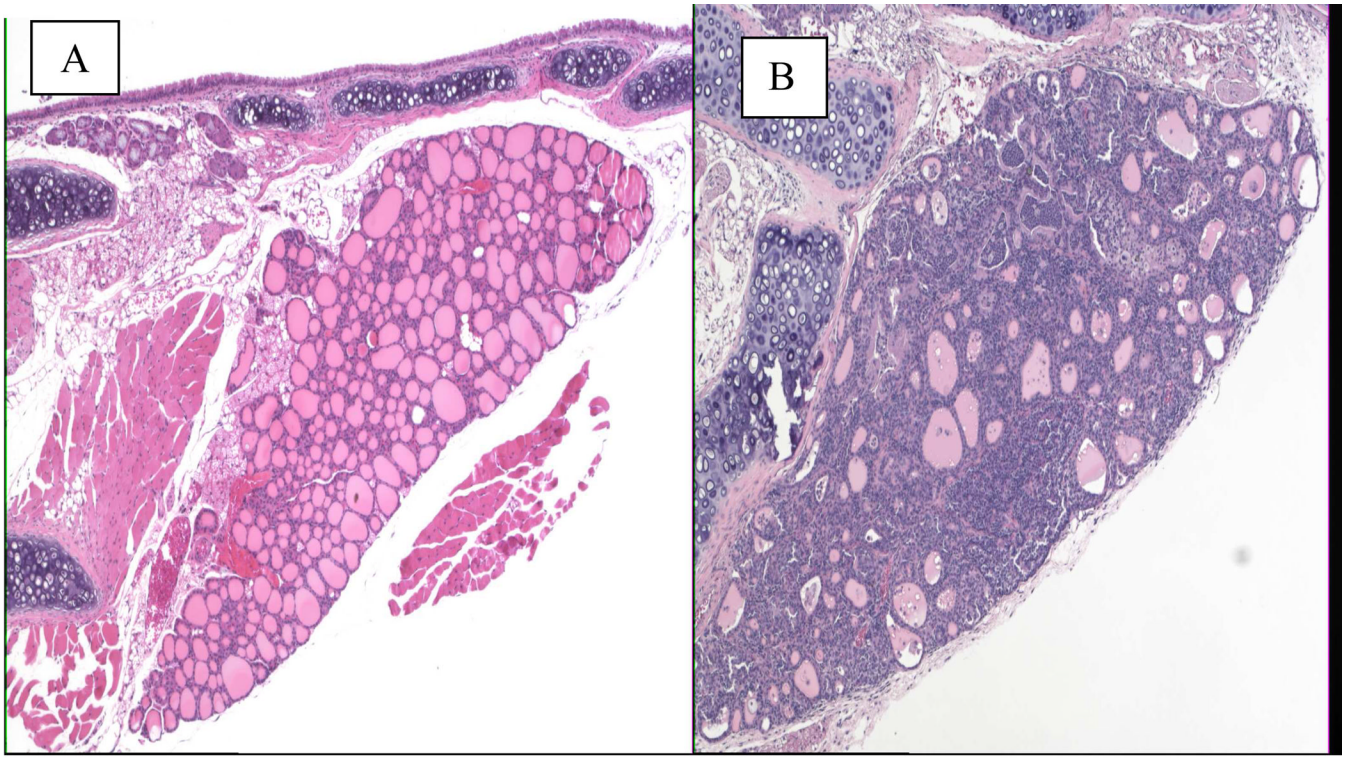

Figure 1.
Thyroid glands from NOD.H2^{h4} mice. A. normal unaffected gland from an untreated mouse. B. Infiltrated gland (+4) after 8 weeks of treatment with excess iodine in the drinking water.

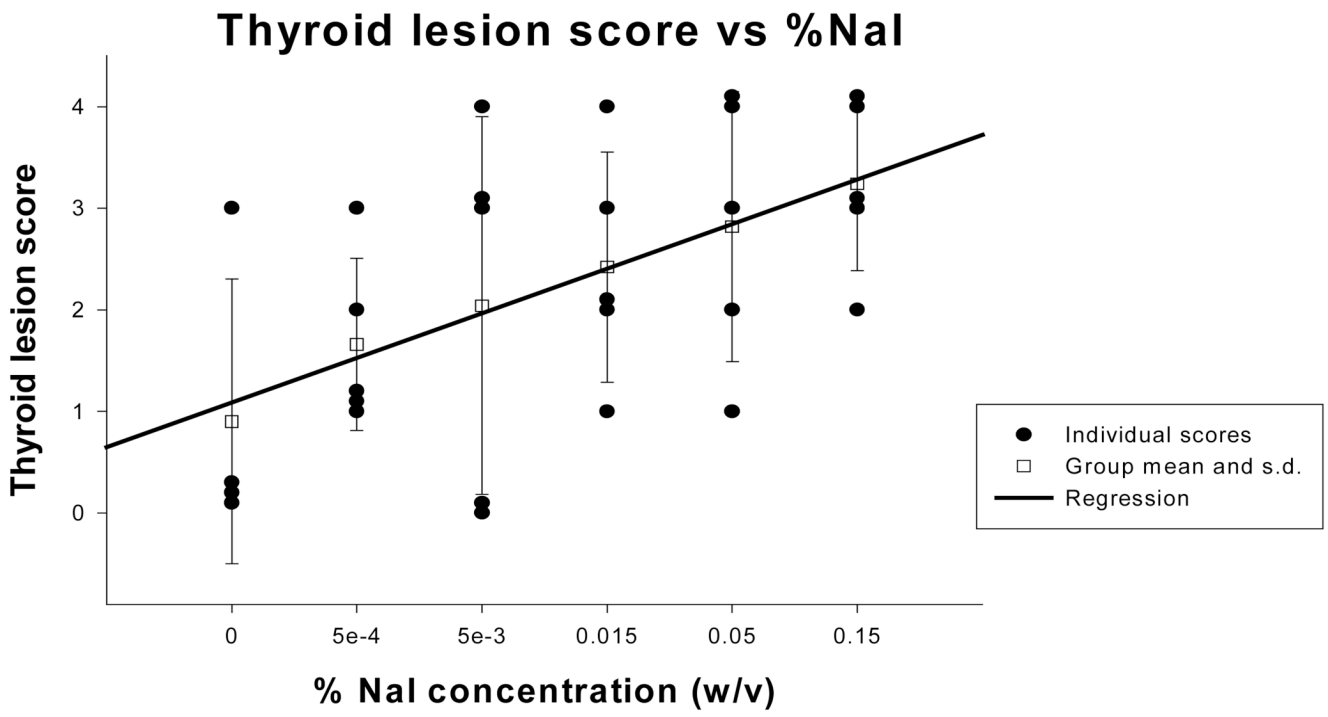

Figure 2.

Dose response of NaI concentration and thyroid lesions in NOD.H2^{h4} mice born and bred in conventional housing. The thyroids are graded on H and E stains from paraffin sections. Six to 8 sections of tissue are examined. Two independent readers evaluate the tissue. The thyroids are scored for infiltration by the following criteria:

0 = no infiltration,

1+ = infiltration in <20% of the gland,

2+ = infiltration from 20–30% of the gland,

3+ = infiltration from 30–50% of gland,

4+ >50% infiltration of gland

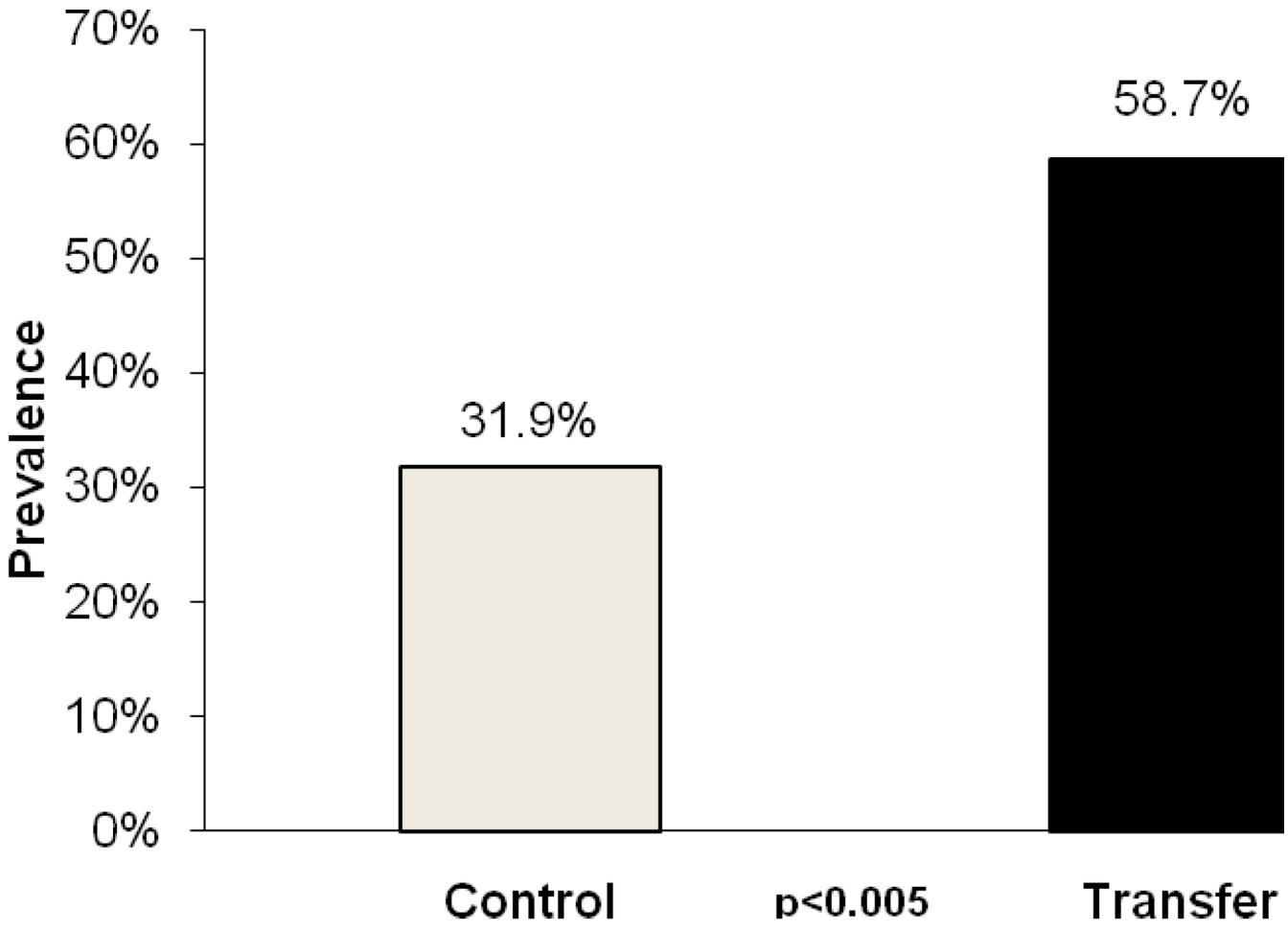

Figure 3.

Combined results of three transfer experiments. All donors received 0.15% NaI water for at least 8 weeks. Spleen cells were cultured for 3 days with 25 ug/ml of mouse thyroglobulin. Transfer group recipients (n=63) received low dose iodine (0.005%w/v) two weeks prior to transfer of spleen cells given i.v. Control mice (n=69) similarly on low dose iodine received PBS instead of cells. Thyroids were assessed for lymphocytic infiltration 14 days after transfer. Conclusions: Thyroiditis can be transferred in this model using spleen cells from mice fed with iodine.

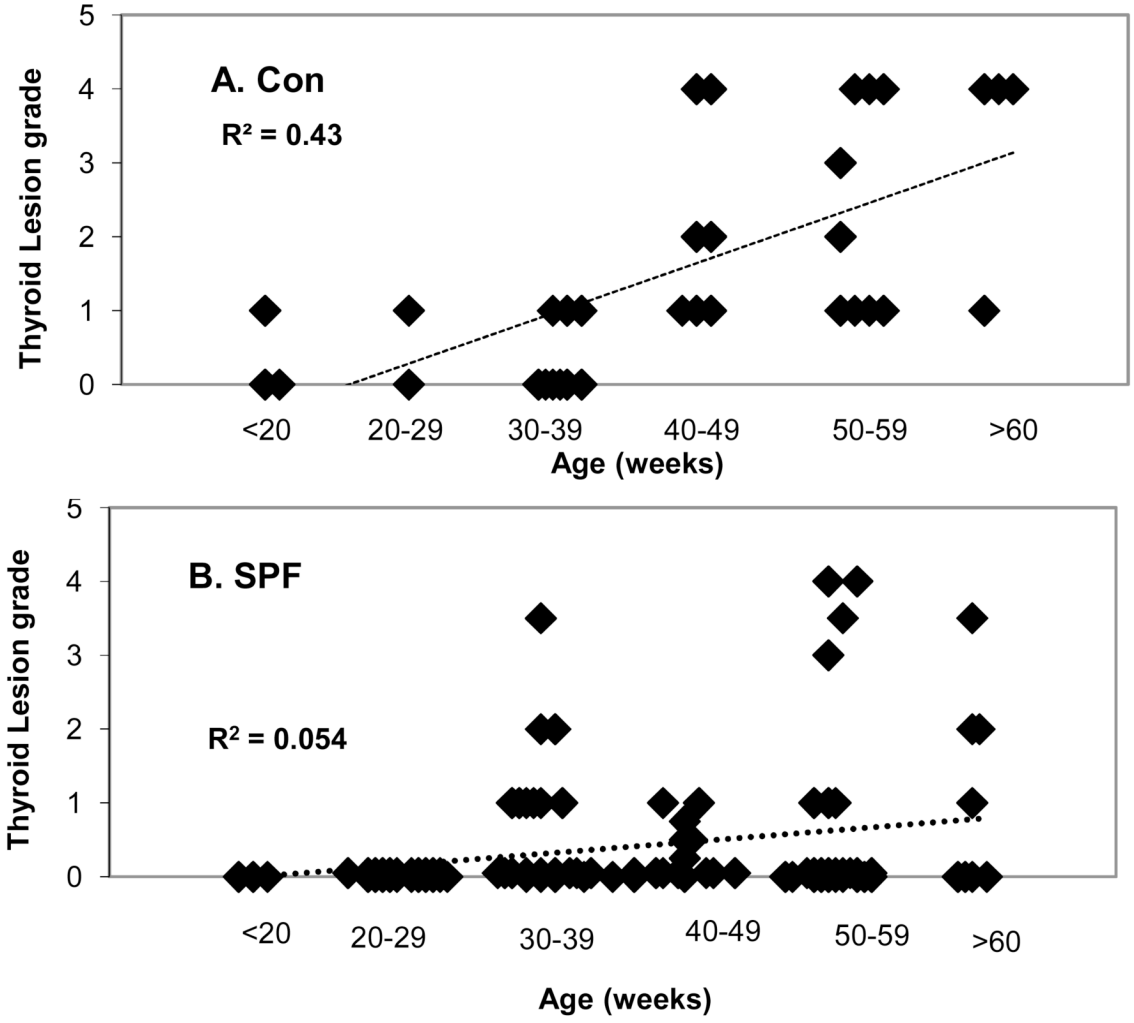

Figure 4.

Frequency of thyroiditis in untreated NOD.H2^{h4} mice housed in conventional (Con) or specific pathogen free (SPF) conditions. A. Mice housed in conventional conditiions developed earlier and more severe thyroiditis (R^2= 0.45 than B. NOD.H2h4 mice born and reared under SPF conditions (R^2=0.054). Both groups showed increased frequency of thyroiditis with age.

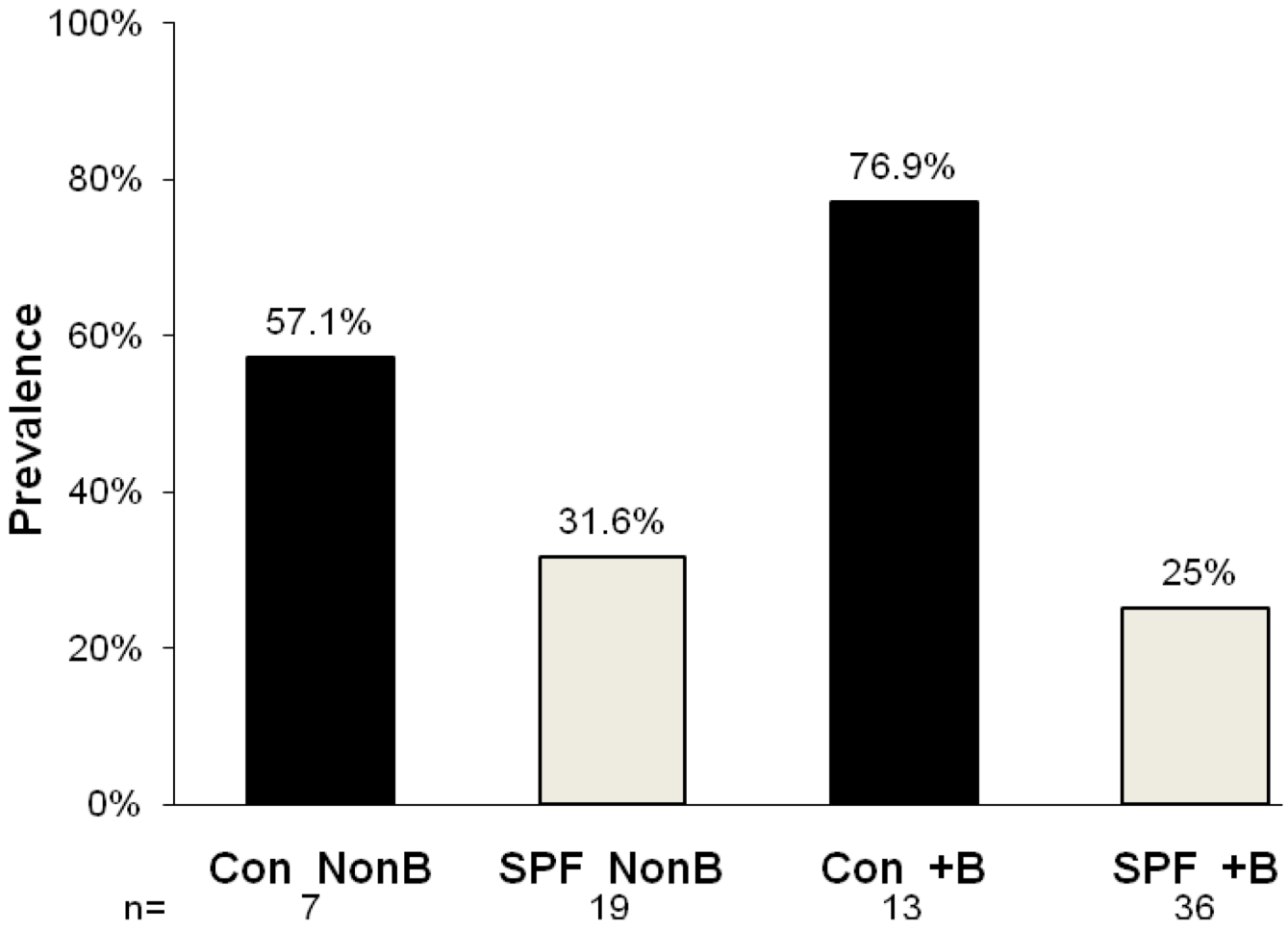

Figure 5.

Prevalence of thyroiditis in female breeder (+B) or non-breeder (NonB) NOD.H2^{h4} mice born and raised in conventional (Con) or specific pathogen free (SPF) conditions. Both breeder and non-breeder groups raised in conventional conditions had a higher frequency of thyroiditis than their SPF counterparts. Conventionally raised breeder mice showed significantly greater thyroiditis over breeder mice raised under SPF conditions ($p<0.03$, Fisher's exact test).

Table 1

Increased risk of thyroiditis in NOD.H2^{h4} mice after different environmental exposures

Housing	Treatment	Odds Ratio
SPF	Bromine	1.5
Conventional	Bromine	3.89
Conventional	LPS	3.9
Conventional	Methylcholanthrene	5.67
SPF	Iodine	6.67
Conventional	Iodine	15.3

LPS 20 ug *Salmonella enteriditis* given twice i.p. at weekly intervals
Bromine 100 mg/l (0.01%)water for 16 weeks
Iodine 0.05% NaI in water for 16 weeks
Methylcholanthrene 0.003g/100 g of chow for 16 weeks
SPF Specific Pathogen Free housing

Odds Ratio over untreated controls calculated according to the formula in Sigma Stat™.