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Early age exposure to moisture damage and systemic inflammation at the age of 6 years

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Abstract

Cross-sectional studies have shown that exposure to indoor moisture damage and mold may be associated with subclinical inflammation. Our aim was to determine whether early age exposure to moisture damage or mold is prospectively associated with subclinical systemic inflammation or with immune responsiveness in later childhood. Home inspections were performed in children's homes in the first year of life. At age 6 years, subclinical systemic inflammation was measured by serum C-reactive protein (CRP) and blood leukocytes and immune responsiveness by *ex vivo* production of interleukin 1-beta (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α) in whole blood cultures without stimulation or after 24 hours stimulation with phorbol 12-myristate 13-acetate and ionomycin (PI), lipopolysaccharide (LPS), or peptidoglycan (PPG) in 251-270 children. Moisture damage in child's main living areas in infancy was not significantly associated with elevated levels of CRP or leukocytes at 6 years. In contrast, there was some suggestion for an effect on immune responsiveness, as moisture damage with visible mold was positively associated with LPS-stimulated production of TNF-α and minor moisture damage was inversely associated with PI-stimulated IL-1β. While early life exposure to mold damage may have some influence on later immune responsiveness, it does not seem to increase subclinical systemic inflammation in later life.

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KEYWORDS
children, cytokines, indoor, inflammation, moisture damage, mold

1 | INTRODUCTION

Indoor dampness and mold in the home environment are a common environmental problem, and a considerable proportion of children and adolescents worldwide are exposed to a moldy and damp home environment.[1,2] Critical reviews and recent epidemiological studies have found increased risks of asthma and respiratory symptoms in children exposed to moisture damage and mold at home in many geographical regions.[3-7]

Until now, the causal agents and the mechanisms behind the association between moisture damage and the adverse health effects are not well known. It has been suggested that microbial components such as cell fragments or spores shed during fungal growth in moisture-damaged buildings may induce inflammatory responses.[2] In this context, a chronic inflammatory response of the respiratory tract is considered as a key factor in the pathogenesis of asthma and allergic rhinitis.[8] Indeed, it has been observed that indoor moisture damage is associated with local airway inflammatory responses.[9-11] We have recently shown in the present cohort that current inspector-observed major moisture damage in the child's main living areas (in the living room, child's bedroom, and/or kitchen) was associated with systemic inflammation in 6-year-old children.[12] However, it is not known whether early exposure to moisture damage and mold can have long-term effects on the systemic inflammation, immunologic responsiveness and thus contribute to the development of inflammatory diseases. The aim of this study was to evaluate whether exposure to moisture damage or mold early in life is prospectively associated with subclinical systemic inflammation (C-reactive protein (CRP) and leukocytes) and immune responsiveness ex vivo (cytokines) in 6-year-old children.

Practical implications

- Subclinical systemic inflammation is associated with several adverse health implications. Here, we show that exposure to moisture damage and mold in the first year of life would not appear to lead to irreversible subclinical systemic inflammation although cross-sectionally such an association has previously been suggested. This finding is valuable for risk assessment and for etiological studies related to moisture damage-associated adverse health effects.

2 | MATERIAL AND METHODS

2.1 | Study population and study area

The study population of 442 children consists of a general population-based birth cohort located in the eastern parts of Finland that has been prospectively followed up from the third trimester of pregnancy and has been described previously in detail.[13] Briefly, the first half of the study population (N = 214) is the Finnish part of the European birth cohort study PASTURE (Protection against Allergy Study in Rural Environments), recruited from 9/2002 to 5/2004 in rural areas.[14] The second half of the study population was recruited between 5/2004 and 5/2005 in mainly sub-urban areas and is its extended cohort (N = 228), using identical methodology.[13] The ethical permission of the study was granted by the Research Ethics Committee of the Hospital District of Northern Savo, Kuopio, Finland. Written consents were acquired from the parents of the participating children.

2.2 | Questionnaires and home inspection

General information about the study population was assessed during the third trimester of pregnancy. The exact methodology of the home inspection has been described earlier and in detail.[7] In short, the homes (N = 413) were inspected by a civil engineer for the signs of moisture and mold in the surfaces and the structures without opening the structures using a predesigned checklist and surface moisture meter (Doser BS2; Doser Messtechnik GmbH & Co., Füssen, Germany) when the children were 5 months old on average. Twenty-nine families could not participate of the moisture damage inspection. During the home visit, housing characteristics were assessed. The parents were informed about the results of the home inspections.

2.3 | Exposure assessment

Moisture damage was originally graded using a 6-point "need for repair" estimation scale in addition to the size of the damaged area A detailed description of the exposure assessment has been comprehensively depicted previously.[7,15] Briefly, "no damage" corresponded to "need for repair" classes 0 and 1 which was defined as no need for repair or only cosmetic repair. "Major damage" included (i) a need for repair class 2 (a repair of surface materials needed) with the area of damage ≥1 m^2, (ii) a need for repair class 3 (a repair of structural components needed) with the area of damage ≥0.1 m^2 or (iii) a need for repair class 4 or 5 (more extensive repair needed). Other damage not described by the characterization above was defined as "minor damage." In the presence of several areas of damage in a given room or area, the areas of damage with the same need for repair estimation were summarized. In addition, the presence of "mold odor" and "visible mold" was recorded for each moisture damage observation. Mold growth which was detectable on silicone sealants in the kitchen or in the bathroom was classified as "no mold."

In this study, we focused on two combined variables describing (i) "moisture damage in the child's main living areas" and (ii) "moisture damage with mold in the child's main living areas" using information regarding signs of moisture damage and signs of moisture damage with mold, respectively, in the child's bedroom, the living room, and/or the kitchen. In additional analyses, we also analyzed the association between exposure to signs of moisture damage and signs of moisture damage with mold in the bathroom and the measured health outcomes, depending on data availability.

2.4 | Health outcome assessment at 6 years

Venous blood samples were collected in lithium-heparin (samples for cytokine analysis), EDTA (white blood cell count), and serum separation (CRP) tubes by an aspiration technique (Vacutainer, Becton&Dickinson, Plymouth, United Kingdom). Samples were stored at room temperature and processed within 27 hours.

Serum samples for the CRP analysis values were frozen (−80°C) and analyzed in a batch by SYNCHRON® System(s) (Beckman Coulter Inc., Fullerton, CA, USA) in Marburg, Germany, according to the manufacturer's instructions. The detection limit was 0.20 mg/L and the CRP levels below the detection limit were set to zero. Children with CRP values over 5 mg/L (n = 10) were excluded from the analyses to exclude children with acute infections. Leukocyte counts were analyzed using Sysmex KX-21N blood cell analyzer (Sysmex Corporation, Kobe, Japan).

Immune responsiveness ex vivo was assessed by measuring cytokine production in stimulated whole blood cell cultures described earlier.[12] In short, heparinized and diluted whole blood was stimulated with the combination of phorbol 12-myristate 13-acetate and ionomycin (PI), or lipopolysaccharide (LPS), or peptidoglycan (PPG) (all from Sigma, Deisenhofen, Germany) for 24 hours. Unstimulated and stimulated cell-free supernatants were frozen (70°C) for later analyses. Proinflammatory cytokines interleukin (IL)-1b and IL-6, and tumor necrosis factor (TNF)-alpha were analyzed using multiplexed cytometric bead array according to the manufacturer's instructions (BD Biosciences, San Jose, CA, USA) in Marburg, Germany. The detection limits were 2.3 pg/mL for IL-1β, 0.7 pg/mL for TNF-a, and 1.6 pg/mL for IL-6 cytokines. In the analyses, non-detected cytokine levels were set to detection level and standardized with leukocytes. Unfortunately, in total, 60 children had to be excluded from the cytokine data as their cell cultures contained serum which was mislabeled by the manufacturer and did not meet the standards (FBS Gold, PAA Laboratories, Pasching, Austria).[12] In this study, 35 children with data on home inspection were excluded from the statistical analyses of cytokines due to mislabeled serum.

2.5 | Statistical analysis

In total, the following data were available for the statistical analyses on the association between early age exposure to moisture damage with or without signs of mold with respect to inflammation markers: 270 children for CRP, 254 for leukocytes, 254 for PI- and LPS-stimulated, 252 for PPG-stimulated, and 251 for unstimulated cytokines. Characteristic analyses were based on the children with complete data on home inspection at early age and measurements either on CRP or cytokines (N = 292). The characteristics of the study population and the buildings had only few significant differences between those who were included (n = 292) and those who were excluded from this study population (n = 150). The included population consisted of more farming families than the excluded participants (31% vs 22%, $P = .05$). Accordingly, the study participants tended to live more often in single-family/semidetached houses (83% vs 75%, $P = .06$) and had greater living area (median 120 m^2 vs 115 m^2, $P = .06$) than those who were excluded from the analyses (29 had missing data on moisture damage investigation and building characteristics). However, there was no difference in the prevalence of early age moisture damage with or without mold in the child's main living area ($P = .54$ and $P = 0.80$) (data not shown).

Binomial logistic regression models[16] were applied to analyze the associations between early age exposure to moisture damage with and without mold and higher levels of subclinical inflammation markers and immune responsiveness. For doing so, the markers were dichotomized into two groups: values below and above the 75th percentile. Further, as the different cytokines stimulated with the same stimulant correlated positively (data not shown),[12] combination variables for IL-1β, IL-6, and TNF-a were created by taking the ranks of the three cytokines within each stimulates. Accordingly, the combined variables were also dichotomized (values below and above the 75th percentile). Due to partly non-sufficient amount of cases and non-significant findings, we decided not to report the results for exposure to moisture damage with and without visible mold assessed in specific locations of the home along with the tested inflammatory markers. To compare our prospective findings with the cross-sectional-based cytokine results in Mustonen and colleagues,[12] we ran additional analyses on early age exposure to moisture damage with and without mold in the bathroom. Moreover, when we tested whether the associations between early age exposure to moisture damage in the child's main living areas and the markers were confounded by current moisture damage exposure in the child's main living areas, the models were additionally adjusted for current exposure at the age of 6 years. However, due to low numbers of observations, we were not able to perform similar additional analyses with moisture damage with visible mold in the child's main living areas, and, in addition, we were not able to analyze the data using continuous exposure (ie, exposure present at both time points).

The main regression models were adjusted for the following a priori selected confounding factors based on earlier findings: gender, living on a farm, older siblings, maternal smoking during pregnancy, maternal allergy, body mass index (BMI), and study cohort. All statistical analyses were performed using the statistical software R, version 3.4.0 (R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.). The present analyses had 78% power with 5% level of significance to detect an odds ratio of 3.1, as earlier reported in a cross-sectional study[12]

comparing prevalence of high CRP among children living in homes with and without major moisture damage.

3 | RESULTS

3.1 | Study population, exposure, and health outcome characteristics

According to Table S1, 31% of the included children grew up in a farm household. During pregnancy, 15% of the study subjects were exposed to maternal tobacco smoking. Over half of the mothers (55%) reported to be affected by an allergic disease. About two-thirds (68%) of the children had older siblings at birth and majority (83%) of the children attended the day care at the age of 1 year or later. The majority of the families lived in detached or semidetached houses (83%). The median size of the living area was 120 m^2. In total, according to the inspector's overall assessment of the whole home during the first year (mean age 5.1 months, standard deviation 5.8), 41% of the homes needed repair of surface materials, whereas 34% were classified as needing a repair of structural components. At the age of 6 years, observed minor moisture damage in the child's main living areas (child's bedroom, living room, and/or kitchen) was recorded in 11% homes, major damage was indicated in 6% homes. Moisture damage with visible mold in the child's main living areas was rare: For mold spots and visible mold, only 1% were indicated, respectively. In total, 32 (11%) children moved during the first 2 years of life, and approximately 36% of the families moved to a different house during the first 6 years of the study period.

As shown in Table 1, early age moisture damage in the child's main living areas classified as "minor" was observed with 24% and major damage was found in 13% of the homes. From the inspected child's main living areas, most of the moisture damage was observed in the kitchen (minor 20%, major 5%). Moisture damage with visible mold was less common: inspector-observed moisture damage with mold spots in 3% of the child's main living areas and 5% of moisture damage with visible mold. Distributions, including the percentiles and the detection limits of the markers of subclinical inflammation and immune responsiveness ex vivo among all available measurements assessed at the age of 6 years, are given in Table 2.

3.2 | Associations between early age exposure to moisture/mold damage and markers of systemic inflammation

Early age exposure to moisture damage with or without mold in the child's main living areas was not significantly associated with CRP levels or number of leukocytes in adjusted models at the age of 6 years (Table 3). No significant associations were found between early age exposure to moisture damage with or without mold in the bathrooms and CRP or leukocytes (data not shown).

3.3 | Associations between early age exposure to moisture/mold damage and immune responsiveness

Early age exposure to moisture damage with visible mold in the child's main living areas was significantly associated with elevated levels of LPS-stimulated TNF-alpha in later childhood (adjusted odds ratio, 95% confidence intervals, aOR (95% CI): 3.72 (1.08-12.83)) (Table 4). In addition, there was an inverse association between the exposure to moisture damage in the child's main living areas classified as "minor" and PI-stimulated production of IL-1β at the age of 6 years (aOR (95%CI): 0.43 (0.19-0.96)).

There were no other consistent associations between early age exposure to moisture damage with or without mold in the child's main living areas and production of the remaining unstimulated and PI-stimulated cytokines (Table 4) as well as their combined variables (Table 5).The analyses were also repeated using selected outcome variables as continuous variables, but the conclusions from these analyses were very similar to those that are presented (data not shown). No associations were found between early age exposure to moisture damage with or without mold in the bathrooms and any analyzed cytokines (data not shown).

3.4 | Sensitivity analysis

We further investigated whether the observed effect of early age exposure to moisture damage in child's main living areas on inflammatory markers at 6 years was independent of the current

TABLE 1 Early age exposure to moisture damage with or without visible mold based on home inspections (N=292*)

	Child's bedroom	Living room	Kitchen	Child's main living area	Bathroom
Moisture damage					
No damage	248 (85%)	238 (82%)	217 (74%)	184 (63%)	108 (37%)
Minor damage	36 (12%)	38 (13%)	59 (20%)	71 (24%)	89 (30%)
Major damage	8 (3%)	16 (5%)	16 (5%)	37 (13%)	95 (33%)
Moisture damage with visible mold					
No mold	280 (96%)	283 (97%)	279 (96%)	269 (92%)	257 (88%)
Only spots	6 (2%)	5 (2%)	5 (2%)	10 (3%)	12 (4%)
Visible mold	6 (2%)	4 (1%)	8 (3%)	13 (5%)	23 (8%)

*Study population: data on home inspections in early age and either available C-reactive protein (CRP) or cytokine measurements at 6 years of age.

TABLE 2 Distributions of C-reactive protein, leukocytes and unstimulated and stimulated cytokines at the age of 6 years

Variable	Unit	N	DL	N (%) values below DL	Percentiles			
					25th	50th	75th	99th
CRP	mg/L	270	0.2	114 (42%)	0.0	0.3	0.7	4.2
Leucocytes (WBC)	10^3/nL	254			5.9	7.0	8.0	12.4
TNF-a								
Unstimulated	pg/(10^9 × WBC)	251	0.7	33 (13%)	0.8	3.0	23.9	674
PI-stimulated	pg/(10^9 × WBC)	254	0.7	0	1420	2340	3690	7020
LPS-stimulated	pg/(10^9 × WBC)	254	0.7	1 (0.4%)	556	775	1220	4500
PPG-stimulated	pg/(10^9 × WBC)	252	0.7	0	1050	1430	2130	6630
IL-1p								
Unstimulated	pg/(10^9 × WBC)	251	1.6	4 (1.6%)	11	56	763	14 500
PI-stimulated	pg/(10^9 × WBC)	254	1.6	0	770	1570	3410	12 900
LPS-stimulated	pg/(10^9 × WBC)	254	1.6	0	10 900	14 700	20 500	62 100
PPG-stimulated	pg/(10^9 × WBC)	252	1.6	0	10 900	15 200	21 800	43 500
IL-6								
Unstimulated	pg/(10^9 × WBC)	251	2.3	34 (14%)	2.6	3.7	20	607
PI-stimulated	pg/(10^9 × WBC)	254	2.3	0	81	143	359	2700
LPS-stimulated	pg/(10^9 × WBC)	254	2.3	0	1140	1730	2600	6580
PPG-stimulated	pg/(10^9 × WBC)	252	2.3	0	2740	3590	4560	9680

N, the number of observations; DL, detection limit; CRP, C-reactive protein; TNF-α, tumor necrosis factor alpha; WBC, the number of white blood cells, that is leukocytes; nL, nanoliter; IL-1β, Interleukin 1-beta; IL-6, Interleukin 6; PI, phorbol 12-myristate 13-acetate and ionomycin stimulated; LPS, lipopolysaccharide stimulated; PPG, peptidoglycan stimulated; pg, picogram.

TABLE 3 Adjusted associations between early age exposure to moisture damage with or without visible mold in the child's main living areas and systemic inflammation markers (CRP and leukocytes) at the age of 6 years

	N	n(%) of CRP ≥75th percentile	aOR (95% CI)	N	n (%) of leukocytes ≥75th percentile	aOR (95% CI)
Moisture damage						
No damage	170	42 (25%)	1	159	43 (27%)	1
Minor	66	18 (27%)	1.08 (0.55-2.12)	63	14 (22%)	0.87 (0.42-1.79)
Major	34	9 (26%)	1.03 (0.42-2.53)	32	12 (38%)	1.81 (0.74-4.46)
Moisture damage with mold						
No mold	247	65 (26%)	1	237	69 (29%)	-[a]
Only spots	10	3 (30%)	1.26 (0.30-5.23)	5	0	-
Visible mold	13	1 (8%)	0.25 (0.03-2.02)	12	0	-

N, number of observations; CRP, C-reactive protein; aOR, adjusted odds ratio; 95%CI, 95% confidence interval.
Models are adjusted for: gender, living on a farm, older siblings, maternal smoking during pregnancy, maternal allergy, body mass index, and study cohort.
[a]could not be estimated.

exposure, by adjusting for the presence of moisture damage in the child's main living areas at the age of 6 years (Table S1). As shown in Table S2A, B, and C, after adjustment for the current moisture damage, only significant association seen was the inverse association between minor moisture damage in the child's main living areas and combined LPS-stimulated cytokines at 6 years (Table S2C), which did not have any effect when the current exposure was not taken account (Table 5).

4 | DISCUSSION

In the current study, inspector-observed early age exposure to major moisture damage in the child's main living areas including child's bedroom, living room, and kitchen was not significantly associated with systemic inflammation markers assessed at the age of 6 years. On the other hand, some immunomodulatory effects were seen. The exposure to moisture damage with visible mold in infancy was

TABLE 4 Adjusted associations between early age exposure to moisture damage with or without mold in the child's main living areas and the production of unstimulated and stimulated cytokines at the age of 6 years

	N	n (%) of TNF-α ≥75th percentile	aOR (95% CI)	n (%) of IL-1β ≥75th percentile	aOR (95% CI)	n (%) of IL-6 ≥ 75th percentile	aOR (95% CI)
Unstimulated							
Moisture damage							
No damage	157	43 (27%)	1	42 (27%)	1	46 (29%)	1
Minor	62	16 (26%)	0.90 (0.44-1.84)	12 (19%)	0.61 (0.28-1.33)	12 (19%)	0.54 (0.25-1.17)
Major	32	5 (16%)	0.51 (0.17-1.51)	9 (28%)	1.00 (0.27-3.69)	6 (19%)	0.44 (0.15-1.31)
Moisture damage with mold							
No mold	235	61 (26%)	-[a]	60 (26%)	-[a]	61 (26%)	-[a]
Only spots	5	0	-	0	-	0	-
Visible mold	11	3 (27%)	-	3 (27%)	-	3 (27%)	-
PI-stimulated							
Moisture damage							
No damage	159	42 (26%)	1	46 (29%)	1	45 (29%)	1
Minor	63	16 (25%)	0.87 (0.40-1.91)	10 (16%)	**0.43 (0.19-0.96)**	14 (23%)	0.60 (0.28-1.28)
Major	32	7 (22%)	0.84 (0.29-2.44)	7 (22%)	0.77 (0.29-2.01)	5 (16%)	0.43 (0.14-1.30)
Moisture damage with mold							
None	237	60 (25%)	1	59 (25%)	1	60 (25%)	-[a]
Only spots	5	1 (20%)	0.59 (0.05-6.69)	1 (20%)	0.70 (0.07-6.67)	0	-
Visible mold	12	4 (33%)	1.43 (0.36-5.70)	3 (25%)	0.94 (0.23-3.74)	4 (33%)	-
LPS-stimulated							
Moisture damage							
No damage	159	38 (24%)	1	42 (26%)	1	44 (28%)	1
Minor	63	18 (29%)	1.20 (0.60-2.39)	13 (21%)	0.73 (0.35-1.53)	17 (27%)	1.05 (0.50-2.19)
Major	32	9 (28%)	1.29 (0.52-3.19)	10 (31%)	1.14 (0.46-2.82)	4 (13%)	0.42 (0.13-1.39)
Moisture damage with mold							
None	237	57 (24%)	1	62 (26%)	1	62 (26%)	-[a]
Only spots	5	2 (40%)	2.12 (0.33-13.65)	1 (20%)	0.65 (0.07-6.23)	0	-
Visible mold	12	6 (50%)	**3.72 (1.08-12.83)**	2 (17%)	0.66 (0.13-3.20)	3 (25%)	-
PPG-stimulated							
Moisture damage							
No damage	159	40 (25%)	1	41 (26%)	1	42 (26%)	1
Minor	62	16 (26%)	1.07 (0.53-2.14)	15 (24%)	1.07 (0.52-2.19)	17 (27%)	1.26 (0.62-2.57)
Major	31	8 (26%)	0.99 (0.39-2.53)	8 (26%)	1.13 (0.44-2.91)	5 (16%)	0.62 (0.21-1.85)
Moisture damage with mold							
None	235	56 (24%)	1	59 (25%)	1	59 (25%)	1
Only spots	5	2 (40%)	1.94 (0.31-12.20)	1 (20%)	0.70 (0.07-6.71)	1 (20%)	0.66 (0.06-7.01)
Visible mold	12	6 (50%)	2.93 (0.87-9.86)	4 (33%)	1.79 (0.48-6.64)	4 (33%)	2.02 (0.52-7.79)

N, number of observations; CRP, C-reactive protein; aOR, adjusted odds ratio; 95%CI, 95% confidence interval; TNF-α, tumor necrosis factor alpha; IL-1β, Interleukin 1-beta; IL-6, Interleukin 6; PI, phorbol 12-myristate 13-acetate and ionomycin stimulated; LPS, lipopolysaccharide stimulated; PPG, peptidoglycan stimulated. Models are adjusted for: gender, living on a farm, older siblings, maternal smoking during pregnancy, maternal allergy, body mass index, and study cohort.

[a]could not be estimated.

TABLE 5 Adjusted associations between early age exposure to moisture damage with or without mold in the child's main living areas and combined levels of unstimulated and stimulated cytokines at the age of 6 years

	Combination variable[a] (unstimulated)	
	N	n (%) of cytokines ≥75th percentile	aOR (95% CI)
Moisture damage			
No damage	157	44 (28%)	1
Minor	62	13 (21%)	0.65 (0.30-1.39)
Major	32	6 (19%)	0.63 (0.23-1.77)
Moisture damage with mold			
None	235	60 (26%)	-[b]
Only spot	5	0	-
Visible mold	11	3 (27%)	-

	Combined variable[a] (PI stimulated)			Combined variable[a] (LPS stimulated)		Combined variable[a] (PPG stimulated)		
	N	n (%) of cytokines ≥75th percentile	aOR (95% CI)	n (%) of cytokines ≥75th percentile	aOR (95% CI)	N	n (%) of cytokines ≥75th percentile	aOR (95% CI)
Moisture damage								
No damage	159	46 (29%)	1	40 (25%)	1	159	42 (26%)	1
Minor	63	13 (21%)	0.50 (0.23-1.09)	17 (27%)	1.01 (0.50-2.03)	62	16 (26%)	1.20 (0.59-2.44)
Major	32	5 (16%)	0.43 (0.14-1.30)	9 (28%)	1.29 (0.52-3.20)	31	6 (19%)	0.82 (0.29-2.31)
Moisture damage with mold								
None	237	61 (26%)	-[b]	60 (25%)	1	235	59 (25%)	1
Only spot	5	0	-	1 (20%)	0.76 (0.08-7.10)	5	1 (20%)	0.62 (0.07-5.96)
Visible mold	12	3 (25%)	-	5 (42%)	2.58 (0.75-8.90)	12	4 (33%)	2.02 (0.52-7.82)

N, number of observations; CRP, C-reactive protein; aOR, adjusted odds ratio; 95%CI, 95% confidence interval; TNF-α, tumor necrosis factor alpha; IL-1b, Interleukin 1-beta; IL-6, Interleukin 6; PI, phorbol 12-myristate 13-acetate and ionomycin stimulated; LPS, lipopolysaccharide stimulated; PPG peptidoglycan stimulated. [a]Combination variables for IL-1β, IL-6, and TNF-a were created by taking the ranks of the three cytokines without stimulant (unstimulated) and within each stimulates (PI, LPS, and PPG). Accordingly, the combined variables were also dichotomized. Models are adjusted for: gender, living on a farm, older siblings, maternal smoking during pregnancy, maternal allergy, body mass index, and study cohort.
[b]could not be estimated.

directly associated with ex vivo LPS-stimulated TNF-alpha production at 6 years of age. In addition, moisture damage was inversely associated with *ex vivo* PI-stimulated IL-1β production. Similarly, when taking into account early and current exposure in the child's main living areas, there was a significant inverse association between minor moisture damage and combined LPS-stimulated cytokines at 6 years, but there was no association with systemic inflammation or other markers of immune responsiveness.

In line with our results, a Swedish study (Uppsala) of the prospective ECRHS (European Community Respiratory Health Survey) did not find evidence for an association between questionnaire-assessed moisture damage or mold in workplace buildings in relation to levels of CRP over a follow-up period of 10 years in adults.[17] In contrast to these results from prospective studies, cross-sectional study set among the same study subjects as with the present investigation found a significant association between major moisture damage in child's main living areas at 6 years of age and elevated circulating CRP levels at same age.[12] In addition, higher total fungal contamination in settled dust samples in Swedish day care centers assessed by quantitative PCR was significantly associated with elevated levels of CRP in 62 female teachers.[18] This suggests that immediate effects of exposure to moisture damage are more relevant in relation to systemic subclinical inflammation; however, the importance of early age exposure over a longer time period needs to be confirmed in larger study sets.

Previous work has demonstrated that dampness-related microbes may trigger the production of proinflammatory mediators. Studies with murine cells, as well as studies with human macrophages and lung epithelial cells, have shown that dampness-related microbes or toxins are potent cytokine inducers.[10,19-21] A study investigating ex vivo effects comparing subjects exposed and not exposed to visible mold at home observed slightly elevated levels of leukocytes in the blood of exposed subjects, but no acute inflammation. However, there was a significant increased release of IL-1β and IL-8 after stimulation of whole blood with *Aspergillus versicolor* in the exposed subjects.[22] One Finnish study found that working in

moisture-damaged schools was associated with increased levels of IL-1β, TNF-α, and IL-6 in the nasal lavage fluid compared with the subjects in the control building.[23] Another study evaluated the effect of moisture damage repairs in school buildings on the upper airway inflammatory responses of the occupants. It was demonstrated that the repairs significantly reduced the IL-4 levels in the nasal lavage of the school occupants as compared to the respective levels prior the intervention.[11] In general, until now, evidence is very scarce on the *longitudinal* effects of exposure to moisture damage and mold in relation subclinical inflammation particular in children populations.

Indoor environments harbor a variety of microorganisms including fungi, which along with their secondary metabolites, have been found to be increased in moisture-damaged buildings.[24] In the present study, we found that exposure to moisture damage with visible mold in the child's main living areas at early age, but not in the bathrooms, was inversely associated with the production of LPS-stimulated TNF-alpha in later childhood. Results from this same cohort using 6-year cross-sectional data showed that major moisture damage in the child's main living areas and moisture damage with visible mold in the bathroom increased spontaneous and stimulated cytokine production, particularly PI-stimulated IL-1β and IL-6.[12]

In the present prospective study, we observed significant inverse association between the early age exposure to minor moisture damage and ex vivo production of PI-stimulated IL-1β at 6 years. There was also a significant decrease in combined LPS-stimulated cytokine levels at 6 years for exposure to minor moisture damage in child's main living areas, when additionally adjusted for current exposure. This is in line with the aforementioned 10-year prospective study in adults, where a negative association was observed between building dampness and IL-6.[17] One might speculate whether increased dampness without major damage to the interior might induce the simultaneous proliferation of other microbial sources including bacteria and allergens. "Dust Microbiome" studies have recently shown that exposure to a higher bacterial, but also fungal and allergen diversity in settled dust, was inversely related to allergic sensitization, asthma, and recurrent wheezing in children.[25-28] Knowledge is scarce in relation to microbial toxins as secondary metabolites of fungi associated with indoor dampness and mold. We found previously in the second half of the present study population that none of the 42 detected metabolites (out of measured 333 metabolites) in dust samples collected at the age of 1 year were associated with the development of asthma until the age of 6 years, however, the higher number of metabolites was inversely associated with asthma.[29] A recent study by Korkalainen et al. observed that microbial toxins along with microbial cell wall components can result in considerable synergistic proinflammatory interactions at already low exposure levels.[30] Taken together, our current and previous findings can be seen as surrogates from a rich microbial exposure, which is inversely associated with the development of asthma.[31]

A key strength of this study was the objectively inspector-based report of moisture damage and mold within the homes using standardized methods. Moreover, this is the first prospective study looking at early age exposure to moisture damage and mold in relation to inflammatory markers among children assessed in later childhood. A limitation of the study is that the markers of systemic inflammation and immune responsiveness were determinate only at one time point, even they may fluctuate over time. Also, another limitation was the reduced sample size. However, the study populations included and excluded from the present analyses did not differ in the exposure to moisture damage in the child's main living area at early age. The limitation in numbers was seen in particular in the models looking concurrently at early and current exposure to moisture damage with mold, which restricted the possibilities for further analyses as mentioned earlier. Therefore, the estimates may be unstable due to the low number of children and the results have to be interpreted with caution.

5 | CONCLUSION

In conclusion, early life exposure to mold damage may have some influence on later immune responsiveness; however, it does not seem to increase subclinical systemic inflammation in later life.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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