

Susceptibility to allergic lung disease regulated by recall responses of dual-receptor memory T cells*

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Background: Microbial infections are associated with the initial susceptibility to and flares of asthma. However, immunologic mechanisms whereby infections might alter the asthmatic phenotype are lacking.

Objective: To test the hypothesis that memory T cells specific both for a viral antigen and an allergen could influence the pathogenesis of allergic disease *in vivo*.

Methods: We developed a system in which 2 distinct T-cell receptors coexist on the T-cell surface, 1 specific for a virus and the other for an inhaled antigen.

Results: We show that a population of dual-receptor T cells, polarized through a virus-specific T-cell receptor to contain T_H1 or T_H2 cells, can be reactivated through an unrelated T-cell receptor in recall responses *in vivo*. Quiescent memory cells derived from a T_H1-polarized effector population blocked the development of airway hyperreactivity in a model of allergic lung disease, in association with decreased induction of chemokines and eosinophil recruitment. Conversely, reactivation of quiescent T_H2 cells after inhalation of antigen or virus infection was sufficient to lead to the development of airway hyperresponsiveness and allergic pulmonary inflammation in mice whose lungs were previously normal.

Conclusion: These data provide evidence that dual-receptor memory T cells can regulate allergic disease susceptibility and

suggest that they may play a role in mediating the influence of microbes on asthma pathogenesis. (J Allergy Clin Immunol 2004;114:1441-8.)

Key words: Mucosal antigen, T_H1/T_H2 cells, immunoregulation, allergic lung disease, dual-receptor, mice, airway hyperresponsiveness, airway inflammation, asthma

The prevalence of atopic asthma has markedly increased in the past decade. The rapidity of this increase suggests that environmental factors rather than genetic ones are responsible for this rise in the frequency of the disease.¹ On the basis of substantial epidemiologic evidence, it has been postulated that early life exposures to microbes and/or endotoxin may alter susceptibility to the subsequent development of an atopic phenotype.^{2,3} This hygiene hypothesis suggests that a relatively sterile environment in industrialized, urbanized Western countries has contributed to the recent epidemic of asthma and atopy. However, little is known about specific cellular or molecular mechanisms that could account for an effect of microbial exposures on atopic predisposition or ways in which early life environmental factors could influence later immune responses.

Allergen-induced T_H2 cells play a central role in the pathogenesis of asthma and contribute directly to the development of airway hyperresponsiveness (AHR).^{4,5} The recrudescence of symptoms on exposure to a previous sensitizing antigen is a hallmark of atopic asthma, suggesting that the recall responses of memory T_H2 cells contribute to flares of allergic airway diseases. However, upper respiratory virus infections are the most common cause of exacerbations in atopic patients with asthma,^{6,7} even though viral and other T_H1-oriented infectious challenges very early in life may protect against subsequent development of asthma.⁸⁻¹⁰ The cellular mechanisms that give rise to these influences of microbes on asthma are not known.

Allelic exclusion at the T-cell receptor (TCR) α locus is imperfect, so that T cells can express 2 specificities of functional antigen receptors.¹¹ Thus, a significant percentage (10% to 30%) of mature T cells express 2 TCR α chains and are potentially able to respond to 2 completely unrelated antigens. These observations have suggested that dual-receptor (dualR) T cells may represent special cases in which normal processes of tolerance or immune

*Because memory T cells are sometimes confused with a steady-state CD44^{hi} or CD45RB^{lo} population, which is composed of a variety of subsets including currently activated effector T cells and natural killer T cells, the current work refers to rested (in the absence of antigen), antigen-experienced T cells as memory T cells, as opposed to (currently activated) effectors.

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Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
DualR: Dual-receptor
HNT: Influenza A hemagglutinin
IP: Intraperitoneal
TCR: T-cell receptor

regulation are modified.^{12,13} However, it is unknown whether memory cells derived from dualR T cells in response to stimulation through one TCR can later participate in a recall response to an independent antigen, nor is it clear whether such recall responses could alter disease pathogenesis. We hypothesized that substantial clonal expansion and the commitment of dualR T cells toward T_H1 or T_H2 cytokine production profiles could occur through a microbe-specific TCR, leading to a biased pool of memory T cells. Recall responses of these cells could later influence the susceptibility to allergic airway disease provoked by an allergen unrelated to the microbe.

METHODS**Animals**

DO-11.10 mice (BALB/c) transgenic for a TCR recognizing ovalbumin peptide 323-339¹⁴ and influenza A hemagglutinin (HNT) mice (BALB/c) transgenic for a TCR recognizing HNT peptide from the influenza A hemagglutinin protein¹⁵ were bred in the Vanderbilt University Medical School or in the Cleveland Clinic Foundation mouse facility. Immunocompetent, nonirradiated BALB/c transfer recipients were females 4 to 8 weeks old from Jackson Laboratory (Bar Harbor, Me). All mice were maintained in specific pathogen-free conditions by using microisolator cages and were used in accordance with applicable regulations after institutional approval.

Differentiated CD4⁺ effector T-cell populations and adoptive transfers

CD4⁺ T cells from spleen and lymph nodes of TCR transgenic mice were isolated by negative selection by using anti-CD8 and anti-MHC II microbeads (Miltenyi, Auburn, Calif) and were maintained and polarized under T_H1 and T_H2 conditions as previously described.^{16,17}

Sensitization and lung analyses

For induction of allergic airways disease after aeroallergen inhalation, 1.25×10^6 to 5×10^6 activated cells were transferred via retro-orbital plexus, as indicated, followed by 24 days in the absence of antigenic stimulation. A standard sensitization model of allergic airways disease, anesthesia, lung physiology measurements, and bronchoalveolar lavage (BAL) was performed as described.¹⁸ For infection with influenza virus, mice bearing adoptively transferred T-cell populations were anesthetized with intramuscular ketamine 40 μ g/g and xylazine 6 μ g/g, and 30 μ L PBS containing a nonlethal dose of the H1N1 strain A/PR/8/34 (as a fresh 1/30,000 dilution from a once-thawed stock originally titered as 3×10^6 egg infectious doses per microliter) was administered intranasally. Statistical analyses of differences between groups were performed

with InStat software (GraphPad, Sorrentino, Calif) by using unpaired, nonparametric, 2-tailed *t* testing.

A more detailed Methods section can be found in the Journal's Online Repository at www.mosby.com/jaci.

RESULTS**DualR memory T_H1 cells can abrogate AHR when present before a T_H2-inducing stimulus**

Mice transgenic for the ovalbumin-specific DO-11.10 TCR, identified by using the anticonotypic antibody KJ1-26, were mated with animals expressing an I-A^d-restricted TCR recognizing the influenza hemagglutinin peptide HNT, identified by using the V β 8.3 TCR chain.^{14,15} This receptor pair was physically compatible at the cell surface, as evidenced by flow-cytometric staining (see Fig E1, A, in the Journal's Online Repository at www.mosby.com/jaci), and no apparent abnormalities of thymic selection could be detected (data not shown). Experiments in which T cells were first activated by one peptide, and then restimulated with the peptide ligand for each TCR, indicated that the receptors also were functionally compatible and caused no apparent shift in the effector characteristics of the activated population (see Fig E1, B, in the Online Repository at www.mosby.com/jaci). We conclude that populations of these dualR cells polarized toward a T_H1 or T_H2 profile respond in accordance with the effector status when restimulated through a different TCR.

To test whether a pre-existing population of resting, memory T cells derived from a T_H1-polarized population could affect the development of a subsequent T_H2 response, T_H1 and T_H2 populations were prepared from dualR TCR transgenic mice by activation through the virus-specific TCR and then transferred into naive, immunologically normal BALB/c recipients (Fig 1, A). These activated cells were deprived of antigen during a 24-day period *in vivo* (parking). As in an extensive literature on memory T cells, most (~90%) of the activated effector population died, but some entered a quiescent state, leaving only a small population of memory T cells.¹⁹⁻²² Mice were then subjected to a standard sensitization protocol that leads to allergic lung inflammation and AHR.^{18,23} Whereas AHR was readily detected in control sensitized mice (intraperitoneal aerosol [IP-aero]), the presence of dualR memory T cells polarized toward the T_H1 program after activation through a virus-specific TCR abrogated the ability to develop AHR in this recall-dependent model (Fig 1, B; dualR-T_H1-IP-aero). In contrast, when a population of T_H2 cells derived from the dualR mice was allowed to become quiescent in naive recipient mice, robust AHR developed (Fig 1, B; dualR-T_H2-IP-aero).

Decreased allergic inflammation and chemokine RNA induction in mice bearing a T_H1-derived memory subset

Allergic inflammation as measured by eosinophil recovery in BAL fluid was significantly reduced (Fig 2, A;

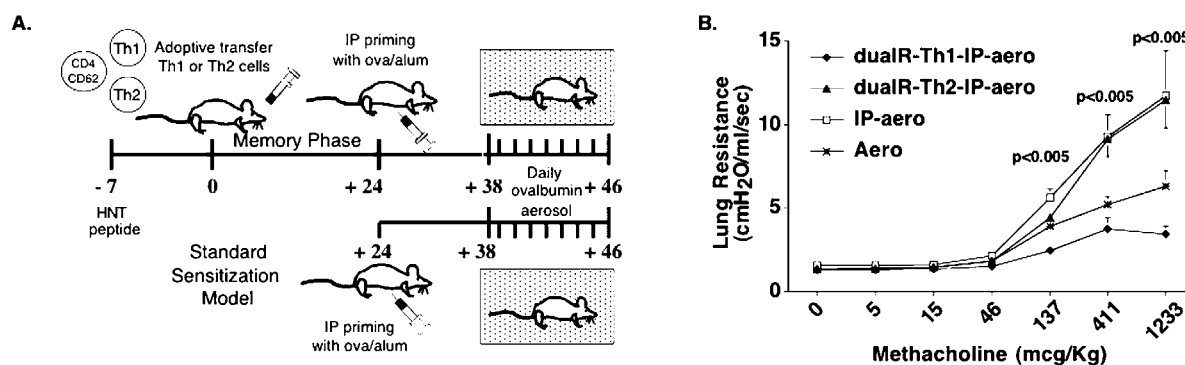


FIG 1. A dualR memory T_H1 population originally primed by a virus-derived peptide abrogates airway hyperreactivity in response to unrelated allergen. **A**, Schema of experimental protocol. Naive ($CD4^+CD62L^{hi}$) cells were purified from DO-11.10 $\times$ HNT double transgenic mice (dualR) and cultured under T_H1 or T_H2 polarizing conditions with HNT peptide and antigen-presenting cells as detailed in the Methods section, after which the indicated populations were transferred into nonirradiated BALB/c recipients (day 0). Twenty-four days after cell transfer, mice received an IP injection of 10 µg ovalbumin in alum, followed by 8 sequential ovalbumin inhalation sessions (days 38–45), identified as dualR- T_H1 -IP-aero or dualR- T_H2 -IP-aero in the figure. As a positive control, a standard sensitization protocol was initiated on day +24 of the memory experiment, as detailed in the Methods section and identified as IP-aero in the figure. Negative control mice received ovalbumin aerosol alone (aero). **B**, Mean ($\pm$ SEM) lung resistance values after methacholine challenge are shown. Each mean value was derived from 6 to 8 individual mice analyzed in 4 separate experiments ($P < .005$ dualR- T_H1 -IP-aero vs IP-aero at doses indicated; $P < .05$ IP-aero vs aero).

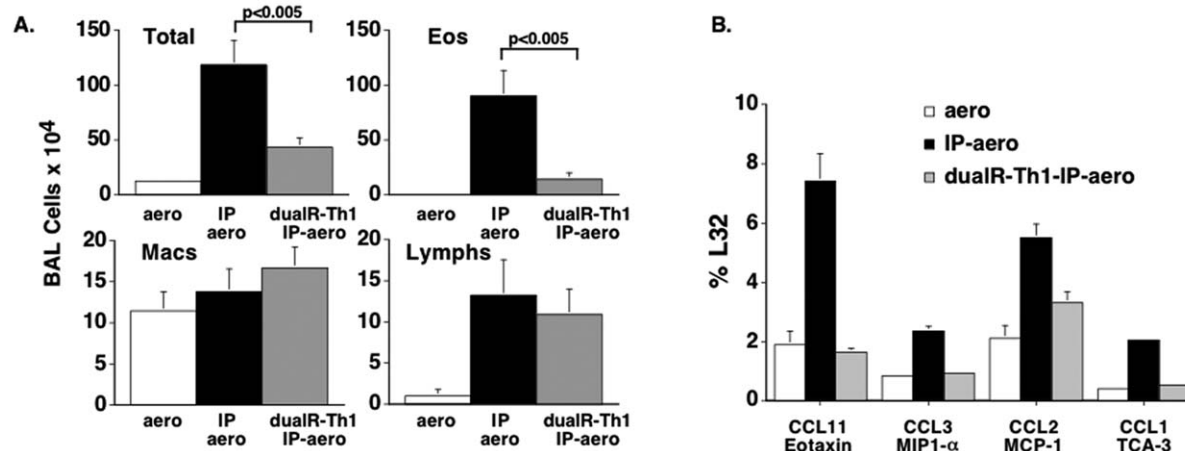


FIG 2. A parked dualR memory T_H1 population originally activated by a virus-derived peptide attenuates allergic pulmonary inflammation in a recall response to inhaled ovalbumin. **A**, Inflammatory cell recoveries in BAL fluid. Data represent mean ($\pm$ SEM) values for the numbers of cells recovered from 6 to 8 individual mice per data point in 4 separate experiments. **B**, Alterations in the antigen-dependent induction of chemokines. Shown are data from ribonuclease protection assays of whole-lung RNA, in arbitrary image units normalized to the L32 internal control mRNA. Data represent mean ($\pm$ SEM), 3 mice per group, from 1 representative experiment of 2 with similar results. Macs, Macrophages.

$P < .005$). Despite the complete abrogation of AHR, there was still a modest recovery of eosinophils from the BAL from the recipients of dualR T_H1 memory cells (dualR- T_H1 -IP-aero) compared with control sensitized mice (14.5 ± 5.5 vs 89.9 ± 23.3 , respectively). Transfer of virus-specific T_H2 cells, parking to allow formation of a memory subset, and subsequent sensitization of recipients with intraperitoneal ovalbumin in alum had a dramatic increase in total BAL cells as well as eosinophils (total cells, 261.9 ± 59.2 ; eosinophils, 193.3 ± 38.6). In addition, HNT single TCR transgenic mice

polarized to a T_H1 or T_H2 phenotype with viral peptide did not alter inflammation in this model (data not shown). To investigate potential mechanisms leading to a decreased number of inflammatory cells, ribonuclease protection assays were performed on the lungs from mice harvested on day 46 (Fig 2, B). The levels of CCL11 (eotaxin), CCL3 (MIP1 α), and CCL1 (TCA-3) in dualR- T_H1 mice were similar to those of negative controls that only inhaled antigen but had not previously been sensitized to ovalbumin (aero), whereas substantial induction of these chemokines was observed in

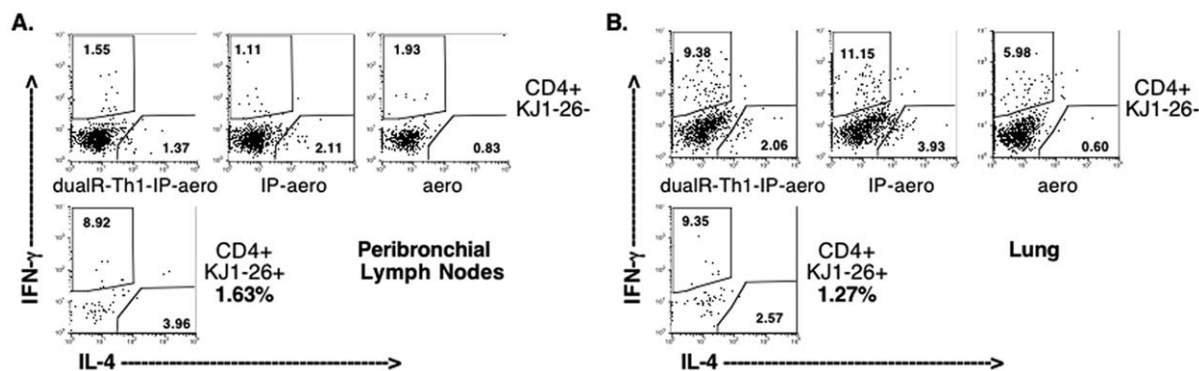


FIG 3. Cytokine production profiles of donor and recipient CD4 T cells. Groups of mice as described in Fig 1 were sacrificed 1 day after the last ovalbumin inhalation, and the peribronchial lymph nodes (A) and lungs (B) were processed for intracellular cytokine staining. Representative data are shown from 1 of 2 separate experiments with similar results (≥ 2 mice per recipient group in each experiment). Also shown are the percentages of cells that were donor-derived (KJ1-26⁺CD4⁺).

lungs of positive control mice which did not bear dualR T_H1 cells before initial allergic sensitization (Fig 2, B; IP-aero). In contrast, induction of the chemokine (CCL2) MCP-1, which may have a role in the development of T_H2 effectors,²⁴ was only modestly reduced by the presence of dualR memory T_H1 cells.

Donor-derived population of rested T_H1 cells can bias cytokine production profiles of the endogenous CD4 subset

To characterize the repertoire and functional characteristics of lymphocytes recruited after aerosol inhalation, lung tissue and peribronchial lymph nodes were harvested and analyzed by fluorescence-activated cell sorting after intracellular cytokine staining (Fig 3, A and B). Despite the dramatic reduction in AHR and eosinophils in the mice that received dualR T_H1 cells, only a small portion of the CD4⁺KJ1-26⁺ T_H1 cells were recovered from the peribronchial lymph nodes and lung (Fig 3, A and B, lower panel). The majority of the recovered, cytokine-producing CD4⁺ KJ1-26⁺ T_H1 cells were IFN- γ ⁺, whereas some (4.0% in peribronchial lymph nodes and 2.6% of lung lymphocytes) were IL-4⁺. Because there were no IL-4-producing donor cells at the time of transfer (see Fig E1, B, in the Online Repository at www.mosby.com/jaci), these donor-derived T_H1 cells must have arisen from activated but uncommitted donor cells in the transfer pool, presumably developing at the time of intraperitoneal priming with ovalbumin in alum. T cells from the naive, endogenous (CD4⁺KJ1-26⁻) repertoire were activated, trafficked to the lung, and developed a T_H2 cytokine profile despite the presence of donor T_H1 cells (Fig 3, A and B, upper panel). However, a decrease in the percentage of T_H2 cells and an increase in the ratio of recipient-derived IFN- γ ⁺ to IL-4⁺ cells were notable distinguishing characteristics between the dualR-T_H1-IP-aero group versus the IP-aero group (see Table E1 in the Online Repository at www.mosby.com/jaci). This finding suggests that the donor T_H1 cells had an effect on the

subsequent immune response to the intraperitoneal priming immunization. The data from Fig 3 indicate that delivery of antigen (ova) in a potent adjuvant (alum) reactivated quiescent memory T_H1 cells whose original activation occurred through a virus-specific TCR. A consequence of this reactivation was a reduction in the development and/or recruitment of cells of T_H2 phenotype from among the polyclonal endogenous repertoire of recipient T cells.

Allergic susceptibility to ovalbumin inhalation generated by dualR T_H2 cells originally activated by a viral antigen

We hypothesized that a population of memory T_H2 cells that were polarized after encounter with a viral antigen would exhibit a recall T_H2 response if they were then reactivated by inhaled antigen specific for a second, unrelated TCR coexpressed on the same T cells. This might thereby constitute another mechanism by which bispecific memory T cells could influence susceptibility to allergic airway disease. T_H1 and T_H2 populations were prepared from the TCR double-transgenic mice by activation through the virus-specific TCR after a single cycle of culture under polarizing conditions and transferred into recipient mice. After the cells had been parked 24 days, mice were exposed to serial inhalations of nebulized OVA in parallel with negative controls (no cells transferred; Fig 4, A, upper panel). As a positive control, a standard sensitization protocol was used (Fig 4, A, lower panel). Little AHR developed if the mice did not receive cells or a priming injection of OVA in alum (Fig 4, B, aero). In contrast, mice that received antigen-specific T_H2 populations developed a dose-dependent increase in reactivity to bronchoconstrictor. The response observed at 2.5×10^6 cells transferred 24 days previously was similar to that obtained with the conventional regimen or with 5×10^6 cells transferred (Fig 4, B). A statistically significant increase in AHR was observed with as few as 1.25×10^6

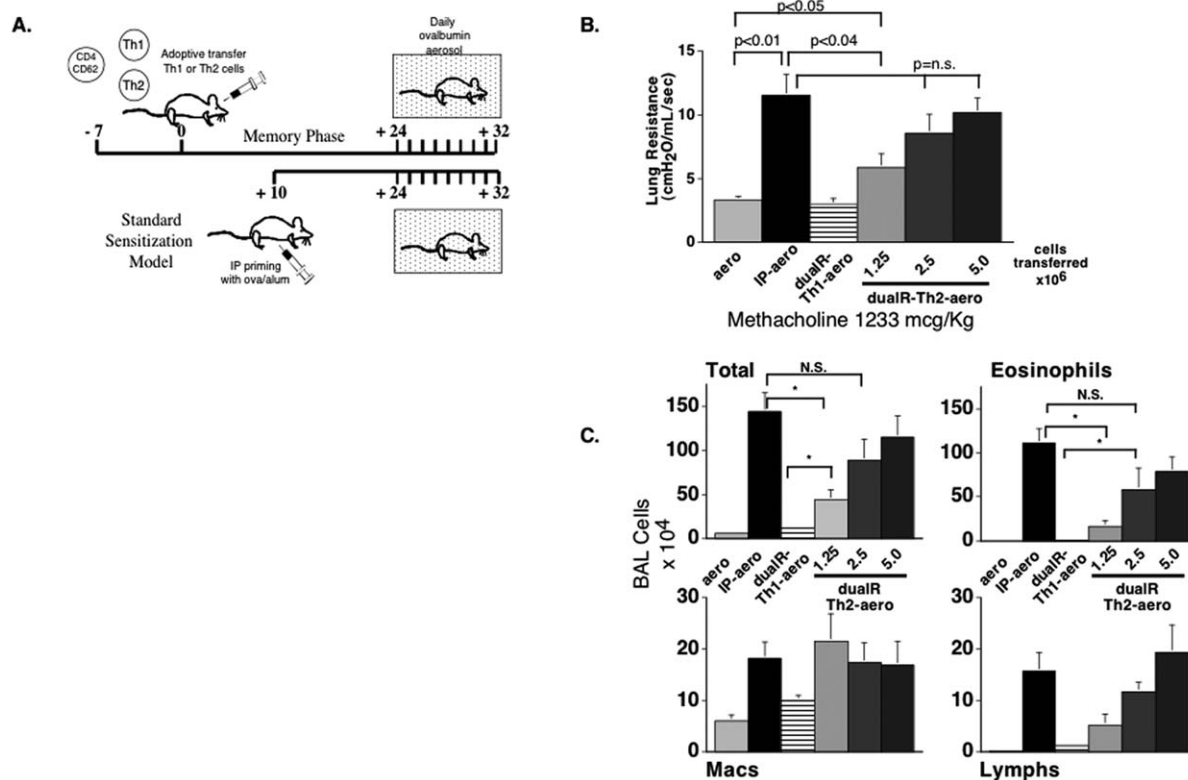


FIG 4. DualR memory T_H2 cells originally activated by a virus-derived peptide lead to allergic airways disease and hyperreactivity after activation of a recall response by ovalbumin inhalation. **A**, Schematic experimental protocol. Naive CD4⁺ T cells were purified from double-transgenic mice and cultured under T_H1 or T_H2 polarizing conditions with HNT peptide and BALB/c antigen-presenting cells. The indicated populations were transferred into nonirradiated BALB/c recipients, and 24 days after cell transfer, mice were subjected to a series of 8 ovalbumin inhalations. As described in Fig 1, A, a standard sensitization protocol was used as a positive control. **B**, Each bar represents the mean ($\pm$ SEM) lung resistance derived by averaging the peak airway response (reproducibly at the dose of 1233 μ g methacholine per kilogram body weight). Each mean was derived from 6 to 8 individual mice in 4 separate experiments. **C**, Cell counts recovered in BAL fluid. Data represent mean ($\pm$ SEM) values for the numbers of cells recovered from the airspace derived from 6 to 8 individual mice in 4 separate experiments. *Macs*, Macrophages. * $P < .05$.

cells transferred. In contrast, parking ovalbumin-specific T_H1 cells did not confer sensitization to inhaled allergen. The transfer of naive cells was unable to confer sensitization to inhaled antigen (data not shown). Further, the dualR memory T_H2 cells led to recruitment of inflammatory cells to the lung parenchyma and air spaces in a dose-dependent manner, whereas naive cells did not (Fig 4, C, and data not shown). These data show that a population of antigen-specific memory T_H2 cells that were stimulated initially with a virus-derived peptide can regulate susceptibility to AHR when reactivated through a second, unrelated TCR.

Enhanced AHR of mice on influenza virus infection generated by dualR T_H2 cells originally activated by ovalbumin

To investigate whether dualR T_H2 cells could influence the magnitude of airways reactivity during virus infection, T cells were activated by using ova³²³⁻³³⁹, cultured under

T_H2 conditions, introduced into normal BALB/c recipient mice, and rested for 4 weeks (Fig 5, A). Mice that received a T_H2 population bearing only the DO11.10 TCR (DO11-T_H2-flu) and mice that received naive dualR T cells (dualR-naive-flu) exhibited a modest increase in bronchial reactivity after intranasal inoculation with influenza related to flu infection (Fig 5, B). Intranasal inoculation with influenza virus led to a significant enhancement of AHR in mice that had received dualR T_H2 cells (dualR-T_H2-flu) compared with those that received either the DO11-T_H2 population or the naive dualR T cells (Fig 5, B). To confirm that dualR T cells were present in the lung after infection with the PR8 strain of influenza virus used in these experiments, the number and percentage of cells from lung homogenates with or without flu infection was determined by cell count and fluorescence-activated cell sorting analysis (see Fig E2, A, in the Online Repository at www.mosby.com/jaci). There was an increase in both the percentage and total number of CD4⁺/KJ1-26⁺ cells recovered in mice that received dualR-T_H2 cells and were

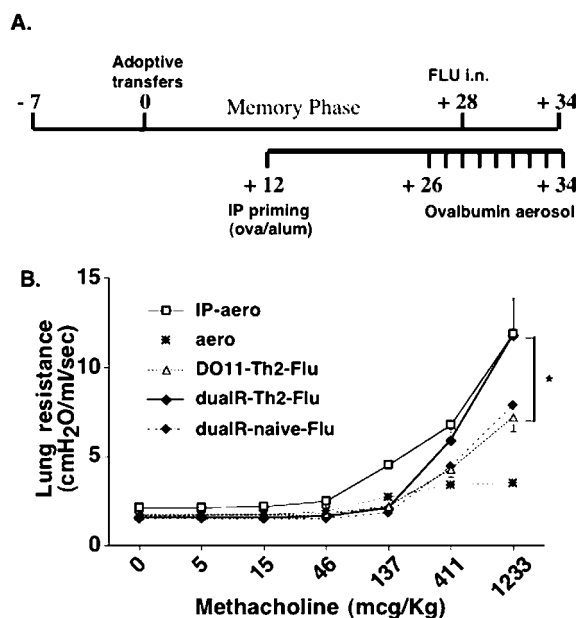


FIG 5. Enhanced AHR of mice bearing a rested, ovalbumin-activated dualR T_H2 population on influenza virus infection. **A**, Schematic of protocol. BALB/c mice received adoptive transfers of equal numbers (4×10^6 cells/mouse) of the indicated populations of T cells, or PBS alone. Activated, T_H2 -polarized effector populations of single transgene DO11.10 T cells (DO11.10 T_H2) and T_H2 -polarized doubly transgenic, DO11.10 $\times$ HNT T cells (dualR T_H2), were transferred into BALB/c recipients. Four weeks after adoptive transfers, mice (2 experimental sets with 2 mice per set) were infected with influenza A/PR/8/34 by intranasal (*i.n.*) inoculation, and a methacholine challenge was performed 6 days after inoculation. As described in Figs 1 and 4, a standard sensitization protocol was used as a positive control. **B**, Mean ($\pm$ SEM) lung resistance values after doses in a series of methacholine injections are shown. IP, Intraperitoneal. * $P < .05$ for IP-aero or dualR- T_H2 -Flu compared with DO11- T_H2 -Flu or dualR-naive-Flu.

challenged with flu compared with mice that received dualR- T_H2 cells with no flu (dualR- T_H2 -no recall; see Fig E2, A, in the Online Repository at www.mosby.com/jaci). Importantly, mice that received DO11- T_H2 cells and were subsequently challenged with flu showed no increase in cell number or percentage of $CD4^+/KJ1-16^+$ cells, showing both that the response was specific to the dualR cells and that these cells survived and responded after parking for 28 days. Carboxyfluorescein diacetate, succinimidyl ester label dilution provided additional confirmation and revealed evidence of cell division of $CD4^+/KJ1-26^+$ cells in the spleen, lymph nodes, and lung after infection with the PR8 strain of influenza virus (see Fig E2, B, in the Online Repository at www.mosby.com/jaci). Together, these findings provide evidence supporting the possibility that exposures to microbial antigens could determine susceptibility to immune-mediated diseases or later asthma flares through the development of memory $CD4$ T-cell populations biased toward T_H1 or T_H2 properties and the subsequent recall responses of these cells.

Finally, to confirm that dualR T cells were present at the time of antigen rechallenge, lymph nodes were harvested 4

weeks after adoptive transfer of *in vitro* polarized T_H2 cells from DO11.10 or dualR mice. Fig E3 (in the Online Repository at www.mosby.com/jaci) reveals that the background of the V β 8.3 TCR on the endogenous $CD4^+$ T-cell population is approximately 10%. Gating on $CD4^+KJ1-26^+$ cells from mice that received DO11.10 T cells reveals a background V β 8.3 staining pattern of 3%. In contrast, mice that received dualR T cells show increased expression of V β 8.3 (95%), suggesting that the vast majority of memory cells were of dual specificity.

DISCUSSION

In considering the pathogenesis of asthma, the role of respiratory infections is pertinent because they precipitate most disease flares.^{6,7,25-27} Moreover, microbial infections in childhood may influence the susceptibility to later development of atopic asthma.^{3,4} However, there is a paucity of experimental data directed toward explaining the potential mechanisms of these clinical observations. The current findings provide evidence of one means by which the immunologic repertoire could become biased in terms of allergen-reactive T_H1 and T_H2 characteristics before the first overt exposure to a potential allergen. First, the data establish that inhaled antigen can act on a rested, antigen-experienced set of T_H2 cells that originally were activated by a completely different antigen. This encounter with a new, inhaled antigen could then lead to allergic lung disease, even though the same inhalation did not cause lung disease if the dualR memory T_H2 population was absent. Such findings suggest a model of another mechanism by which microbial antigens could affect asthma, with a subset of T cells specific for both virus and allergen undergoing repetitive expansion and contributing to the memory-phenotype subset. Further, the results with T cells specific for 2 model antigens provide evidence that this mechanism would be able to influence allergic susceptibility or asthma flares. Thus, virus infection could lead to the development of a memory T_H2 cell repertoire that could be reactivated by otherwise innocuous antigens and predispose individuals to the development of allergic airway disease as a by-product of immune responses to such antigens. Together, these findings provide insights into the pathophysiologic capabilities of T cells competent to recognize 2 unrelated antigens because of their expression of 2 different antigen receptor specificities.

Many peripheral T cells may express 2 TCRs because of inefficient allelic exclusion of the TCR α chain,¹¹ leading to a large repertoire of bifunctional T cells. Speculation about the role of dual TCR cells has focused on forms of unconventional recognition such as alloreactivity or autoimmunity.^{12,28,29} However, recent observations have suggested a more beneficial effect by extending the immune repertoire of TCRs specific for foreign antigens.¹³ The number of T cells specific for any given antigen pair (eg, influenza and ragweed) has not been determined, but it is notable that even in young mice, well more than 10% of T cells are of memory phenotype and

apparently arise from exposure to commensal microbes,^{9,30,31} which are able to stimulate T cells repetitively over time. Thus, it may be that some atopic individuals, over time, develop a memory T_H2 population of sufficient size that they are predisposed to an allergic response and AHR once they start to inhale a potential allergen. Alternatively, a memory T_H2 population generated by allergen but also recognizing viral epitopes may influence asthma flares as a consequence of upper respiratory infection with virus (Fig 5).

The results presented here indicate that dualR memory T_H1 populations, if established before an allergen exposure, can block allergic AHR by diminishing the pool of pulmonary T_H2 cells and induction of chemokines (Figs 1 and 2). Additional mechanisms may be relevant to the question of how previous exposures of the immune system to antigens may influence the susceptibility to allergic responses. Recent findings suggest that the newborn immune system may respond in ways that bias recall responses toward T_H2 function and that neonatal mice typically are impaired in the development of T_H1 memory effector function.³² Whether such findings are a basis for asthma in human beings, or for the epidemiologic evidence underlying the hygiene hypothesis, is not yet clear. Further, intercurrent microbial infections might provide a means to bypass the tolerance induction normally imposed by inhalation of soluble protein antigens.³³ Direct study of AHR in a mouse model suggests that if tolerance induction is a mechanism protecting against atopic asthma, virus infection at a precise time before allergen inhalation might lead to interference with the establishment of tolerance.³³⁻³⁵ However, the response to inhaled soluble protein when antigen-specific memory T cells are present indicates that the mechanisms, which prevent allergic inflammation and AHR, are bypassed once populations of memory T cells have developed¹⁶ (Fig 4).

In contrast with the role of T_H2 cells in promoting allergic pulmonary inflammation, the effect of memory T cells on AHR to bronchoconstrictor includes an ability of a T_H1 population to block a sensitization that otherwise would cause allergic inflammation and AHR. Current evidence indicates that there are profound differences between antigen-specific memory T cells (by their nature, resting and remote from antigen exposure) and the activated, cycling population of effector cells specific for the same antigen.^{36,37} However, there has been virtually no direct experimental investigation of the properties of memory CD4 T cells in allergic disease. Evidence of whether T_H1 cells protect against or prevent T_H2 mediated allergic diseases has been conflicting. In some models, T_H1 cells and their IFN- γ production inhibit aspects of the allergic airway response, whereas other data suggest that an activated effector population of T_H1 cells can enhance the initial onset of inflammation if aerosol is delivered shortly after transfer.³⁸⁻⁴³ If well-defined T_H1 and T_H2 effector populations specific for a single antigen were present and had antigen withdrawal together, the recall response of the memory T_H2 cells appeared to be impervious to the T_H1 population.¹⁶ In comparison with

the current findings, these results suggest that the timing for development of the respective populations (T_H1, T_H2) is a critical determinant of their role in allergic pathophysiology. The clinical correlate of such findings would be children with a genetic predisposition to atopy who were exposed to viral infections or whose initial encounters with certain environmental antigens early in life were biased toward an enlarged T_H1 population through concurrent exposures to endotoxin^{2,3,44} later manifesting a lower penetrance of consequent asthma. As a natural extension of the dualR hypothesis, it may also be possible for a population of allergen-primed T_H2 cells to impede antiviral immunity and promote or augment airways disease.

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