TABLE 1

Experimental	Description	Refs.
Human	Freshly isolated NK cells from human PBMC produce IL-5 by stimulation with irradiated MM-170 melanoma cells or JY B-lymphoblastoid cells and rIL-2. NK cells can produce about 8.8-fold more IL-5 after the secondary stimulation culture. IL-5 production is augmented by IL-4.	12
Human	Human PBLs were cultured with irradiated RPMI 8866 cells in the presence of IL-12, which generated NK1 cells (IL-10 and IFN-γ producer), or in the presence of IL-4, which generated NK2 cells (IL-5 and IL-13 producer).	1
Human	IL-2–expanded IFN-γ–nonsecreting NK cells purified from human PBMC produce IL-4, IL-5, IL-13, and IFN-γ after PHA stimulation. Furthermore, NK cells stimulated with IL-4 inhibit IFN-γ production but increase IL-13 production, whereas those stimulated with IL-12 increase IFN-γ production.	13
Human	Human NK cells were cultured *in vitro* in the presence of IL-15 and/or IL-2 from umbilical cord blood hematopoietic progenitors. $CD161^+CD56^+$ NK cells contain two subsets: a $TNF\text{-}\alpha^+/GM\text{-}CSF^+$ subset and an $IFN\text{-}\gamma^+$ subset. $CD161^+CD56^-$ NK cells contain two subsets: $IL\text{-}13^+$ $TNF\text{-}\alpha^+$ $GM\text{-}CSF^+$ $IFN\text{-}\gamma^-$ subset and $IL\text{-}5^+$ subset.	15
Human	The single-cell level analysis was performed. Two non-overlapping $IL\text{-}13^+$ and $IFN\text{-}\gamma^+$ subsets are identified in adult and neonatal NK cells. IL-4 regulates the size of $IL\text{-}13^+$ subset via proliferation, and both IL-4 and IL-13 inhibit the $IFN\text{-}\gamma^+$ subset.	16
Human	About 2% of human peripheral NK cells produce IL-13 in response to IL-2 examined by intracellular analysis. In addition, the NK3.3 cell line rapidly induces new IL-13 mRNA and protein synthesis in response to IL-2. Established human NK clones can produce IL-13 in response to IL-2 or PMA+ Ionomycin.	14
Human	$CD56^{bright}$ NK cells from human PBL produce significant amounts of IL-13 by IL-15+IL-1β or IL-15+IL-18 stimulation. $CD56^{bright}$ NK cells also produce IFN-γ, TNF-β, GM-CSF by PMA + Ionomycin or a variable combination of monokine (IL-12, IL-1β, IL-15, and IL-18) stimulation.	44
Mouse	NK cells from murine splenocytes stimulated with PMA + Ionomycin and IL-2 are differentiated into NK2 cells (IL-5 and IL-13 producer) in the presence of IL-4 and NK1 cells (IFN-γ, IL-10 producer) in the presence of IL-12.	8
Mouse	IL-2–activated liver NK cells from IFN-γ knockout mice express 10-fold more IL-13 and IL-5 mRNA and protein than normal controls following IL-2 treatment *in vitro*. IFN-γ–mediated control of the size of $IL\text{-}13^+$ NK cell subset *in vivo* is suggested.	14
Mouse	IL-2 + IL-18 stimulation strongly induces IL-13 mRNA and protein in NK cells in mice, and the effects are greater in IFN-γ knockout mice.	33
Mouse	NK cells from murine splenocytes cultured with IL-2 produce IL-13, and those cultured with IL-12 and IL-18 produce IFN-γ, IL-10, and IL-13.	41
Mouse	$DX5^+$ splenocytes show an upregulation of IL-13 expression in response to IL-2 + IL-18 stimulation.	40

Disease (model)	Description	Refs.
Human	NK cells in multiple sclerosis in remission are characterized by a remarkable elevation in IL-5 mRNA and a decrease in IL-12Rβ2 mRNA, as well as a higher expression of CD95.	53
Human	T cells from asthma patient expressed high-level CD40L that is mediated by cell/cell contact with asthmatic NK2 cells. NK2 cells generated *in vitro* induce CD40L expression in T cells.	88
Human	Increased NK2 cell number in asthmatic patients. NK cells purified from asthmatic patient PBMCs increased IL-4 and IL-6 mRNA expression and decrease TNF-α, LT, and IFN-γ mRNA expression. Constitutive activation of STAT6 is observed in NK2 clones from asthmatic patients.	87
Human	Freshly isolated NK cells from polyallergic atopic dermatitis (AD) patients spontaneously release higher amounts of IL-4, IL-5, IL-13, and IFN-γ than do NK cells from healthy individuals, although the number of NK cells in peripheral blood is significantly decreased in AD patients.	52
Mouse	Depletion of NK cells selectively reduces the number of infiltrating eosinophils accompanied by a complete loss of IL-5–producing NK cells and significantly reduced levels of peritoneal lavage fluid IL-5.	81
Mouse	Depletion of $NK1.1^+$ cells (NK cells and NKT cells) before immunization inhibits pulmonary eosinophil and $CD3^+$ T-cell infiltration as well as increased levels of IL-4, IL-5, and IL-12 in BALF in a murine model of allergic asthma. This effect is mediated by NK cells but not by NKT cells.	86

NK cell maturation, IFN-γ production, and homing to the liver have been also suggested.[27]

Because IL-12 is required for NK1 cell differentiation, STAT4 seems to be involved in the differentiation of NK1 cells. IL-12Rβ2 is expressed preferentially in NK1 cells.[1,28] We have reported that the expression of the mRNA for IL-12Rβ2 and T-bet is similar in mouse *in vitro* differentiated NK1 and NK2 cells.[8] Thus, although this is likely, we need to await experimental results suggesting STAT4 and T-bet are actively involved in the differentiation of NK1 cells and/or IFN-γ production.

Human NK cells are known to produce IL-10 in response to IL-2.[12,29] We detected the selective production of IL-10 in mouse NK1 cells that were differentiated *in vitro* with IL-2 and IL-12.[8] No IL-10 production was detected in freshly prepared mouse spleen NK cells or *in vitro* differentiated NK0 or NK2 cells. The mechanism underlying the NK1-specific expression of IL-10 is not clear at this time, but both IL-2 and IL-12 are required for the acquisition of IL-10 production in NK1 cells in mouse[8] and human systems,[29] suggesting that some transcriptional factors induced by IL-2R– and IL-12R–mediated signaling in NK cells are responsible for the chromatin remodeling of the IL-10 gene locus and/or IL-10 gene transcription. Another interesting point is that no IL-10 is produced in NK2 cells. It is known that both Th1 and Th2 cells produce IL-10, and the levels are higher in Th2 cells.[30] Similarly, mouse $CD8^+$ Tc1 and Tc2 cells produce IL-10, and the levels of IL-10 production are higher in Tc2 cells (Kimura et al., unpublished observation). Thus, the NK1-specific production of IL-10 appears to be a unique feature of NK lineage cells. It is worth noting that differentiated NK1 cells have the potential to exert immunosuppressive functions in some aspects of immune responses by the production of IL-10.

The exposure of NK cells to type-1–related cytokines such as IL-2, IL-12, and IL-18 results in IFN-γ production.[4] Cytokine costimulation with IL-2 and IL-12,[31] IL-2 and IL-18,[32,33] IL-12 and IL-18,[34,35] or IFN-α and IL-18[36] enhances IFN-γ production. The secretion of type-2 cytokines from NK2 cells is also induced by IL-2.[8] The production of IL-5 from human NK cells has been reported to be IL-2 dependent.[12] Similarly, the induction of IL-13 and IL-5 at the mRNA and protein levels has also been shown to be IL-2 dependent.[14] In *in vitro* differentiated mouse NK1 and NK2 cells, restimulation with IL-2 alone induces significant amounts of IFN-γ or IL-5/IL-13, respectively.[8] This is a unique feature of NK1/NK2 cells and is not observed in established Th or Tc cells, for which TCR stimulation is required for the sufficient production of type-1 and type-2 cytokines. It is possible that the signaling pathways downstream of IL-2R in NK1/NK2 cells are distinct from those in Th1/Th2 cells. A detailed investigation of signaling pathways and inducible transcription factors downstream of IL-2R, IL-12R, and IL-18R among CD4T, CD8T, and NK cells would be interesting and could provide important hints about the unique signaling machinery in NK cells.

In human NK cells, IL-13 enhances IL-2–induced IFN-γ production and cytotoxic activities.[37,38] A recent study suggests that IL-13 shows two types of reactivity when NK cells are costimulated with IL-13 and IL-2: IL-13 enhanced IL-2–mediated IFN-γ production and inhibited IL-2-mediated IFN-γ production.[26] In addition, IL-13 suppresses IL-2–induced cytotoxic and proliferative activities, although less effectively than did IL-4. IL-13 induces the tyrosine phosphorylation of Jak3 and STAT6 in NK cells but induces distinct STAT6-DNA binding complexes that are distinct from those in T cells.[26] Thus, IL-13, unlike IL-4, appears to play multiple roles in cytokine production in NK cells.

IL-18, identified as an IFN-γ-inducing factor, acts on NK cells to produce IFN-γ and enhance NK activity.[39] IFN-γ production is not induced by IL-18 alone, but by IL-18 in synergy with IL-12. In addition, IL-18 has been found to be a potent coinducer of IL-13 production in mouse NK cells in synergy with IL-2, but not with IL-12.[33] This effect is greater in IFN-γ–deficient mice, suggesting a regulatory role of IFN-γ *in vivo*. In addition, the stimulation of $DX5^+$ NK cells with IL-12 and IL-18 results in the production of IL-13.[40] NK cells prepared from murine splenocytes cultured with IL-2 produce IL-13, whereas those cultured with IL-12 and IL-18 induce the production of IL-13 as well as IFN-γ and IL-10.[41] Thus, IL-18 appears to have regulatory roles in the production of both IFN-γ and IL-13.

IV. PHENOTYPIC AND FUNCTIONAL FEATURES OF NK CELL SUBSETS

Human NK cells express CD161, CD56, CD16, and killer cell immunoglobulin-like receptors (KIRs), and mouse NK cells express NK1.1, DX5/CD49b, and Ly49. Using these cell surface markers, developmental stages and functionally distinct subsets of NK cells have been identified. There is an excellent recent review on the development and function of NK cells.[42] Hematopoietic stem cells express none of the above NK cell markers, and committed NK cells become positive for NK1.1 (mouse) and CD161 (human). Moreover, human NK cells can be divided into two subsets: immature NK cells are $CD56^-$ and acquire CD56 expression during maturation. In human peripheral blood, the majority (~90%) of NK cells are $CD56^{low}$ and express high levels of CD16; the rest are $CD56^{high}$ and express $CD16^{low/-}$.[43] $CD56^{high}$ cells produce IFN-γ, IL-5, and IL-13; $CD56^{low}$ NK cells do not produce these cytokines but show higher cytotoxic activities.[42,44] $CD56^{high}$ NK cells are more sensitive to IL-2 during proliferation. Resting $CD56^{low}$ NK cells are more cytotoxic than $CD56^{high}$ NK cells[45]; however, $CD56^{high}$ NK cells also become highly cytotoxic after activation with IL-2 and IL-12.[45–47] These results suggest that the $CD56^{high}$ cell population includes NK cells producing IFN-γ and IL-13, IFN-γ-producing NK1 cells, and IL-5/IL-13–producing NK2 cells. Further studies, such as single-cell level analysis, will help to clarify these points. In mouse, the CD56 gene does not exist, and an alternative cell surface marker, DX5/CD49b,[48] is used for subset identification. This makes comparative studies of NK subsets between human and mouse more complicated.

It has also been recognized that chemokine receptor expression differs between $CD56^{low}$ and $CD56^{high}$ human NK cell subsets. $CD56^{low}$ NK cells show chemotaxis in response to IL-8. $CD56^{high}$ NK cells, in contrast, express high levels of CCR7 and L-selectin, which are important for homing to the lymphnodes.[49] This observation may account for the preferential residence of $CD56^{high}$ NK cells in the parafollucular T cell area of the lymph nodes.[50] It is interesting that $CD56^{high}$ NK cells accumulate in the placenta during pregnancy, pointing to their immunoregulatory roles against semiallogenic fetuses.[51] These results indicate that $CD56^{low}$ and $CD56^{high}$ NK cells contain functionally distinct subsets and that $CD56^{high}$ NK cells modulate immune responses in the lymph nodes by producing various specific cytokines.

The expression pattern of various cell surface molecules was examined in *in vitro* differentiated human NK1 and NK2 cells.[52] Both NK1 and NK2 cells were found to express increased levels of ICOS, CD95, and CD95L compared to freshly prepared NK cells. CD45 RA expression is significantly higher in freshly prepared NK cells than in NK1 and NK2 cells, whereas CD45RO expression is higher in the NK1 subset than in freshly prepared NK or NK2 cells. The expression of NKG2A is significantly lower in the NK2 subset. It has been noted that NK1 cells express higher levels of CD95 than do NK2 cells.[1] Increased levels of CD95 on NK2 cells have been observed in multiple sclerosis (MS) patients in remission.[53]

After *in vitro* activation with IL-2, murine NK cells develop into two distinct subpopulations with differential expressions of IL-12Rβ2.[28] The phenotype is very stable once established. Both subpopulations express a number of NK-associated markers, including NK1.1, DX-5/CD49b, Ly49A, and Ly49C. The expression of IL-12Rβ2 is correlated with the expression levels of an NK inhibitory molecule, Ly-49G2. However, these $IL\text{-}12R\beta2^{high}$ and $IL\text{-}12R\beta2^{low}$ subpopulations are similarly reactive with exogenous IL-12 and respond to IL-12 stimulation with a rapid production of IFN-γ and increased cytolytic activity. No type-2 cytokine production has been detected in either subpopulation.[28] This is an interesting observation, but the functional significance of these two populations has not been established.

Another interesting question is whether cytotoxic function is distinct between the NK1/NK2 subsets. Human IFN-γ-producing NK cells and IL-5/IL-13–producing NK cells purified from PBMC show similar cytotoxic activities.[12,13] Significantly increased cytotoxic activity has been detected in *in vitro* differentiated mouse NK1 cells as compared with NK2 cells; however, no correlation was noted between the levels of cytotoxicity and the levels of IFN-γ production.[8] Mouse NK1 and NK2 cells express similar and substantial levels of the mRNAs for perforin and granzymes.[8]

Thus, there is at present no clear evidence to indicate that the levels of cytotoxic activity differ between the NK1/NK2 subsets.

V. TRANSCRIPTIONAL REGULATION OF IFN-γ PRODUCTION IN NK AND CD8 T CELLS

Substantial work has been carried out on the transcriptional regulation of cytotoxic effector cell function in CD8 and NK cells.[54] NK cells and $CD8^+$ cytotoxic lymphocytes are the two major cytotoxic lymphocyte subsets in the immune system, and both express an interesting and similar set of transcription factors that may regulate the unique cytokine production profiles of these two lineage cells. As stated above, the expression levels of GATA3 and ROG were quite similar in NK cells and CD8 T cells, and their control of type-2 cytokine production in NK2 and Tc2 cells has been suggested.[8,21] Here, transcription factors that may control the production of IFN-γ in NK and CD8 T cells are summarized.

T-bet is a T-box family transcription factor, and its role in IFN-γ production in Th1 cells and NK cells but not CD8 T cells was originally reported by Glimcher and colleagues.[55] However, the production of IFN-γ from OVA-specific OT-I transgenic CD8 T cells with a T-bet knockout background is impaired, and a significant role for T-bet in the regulation of IFN-γ in CD8 T cells under physiological conditions was revealed.[56] The development of spontaneous multiple inflammatory changes in the airway characteristic of asthma was observed in T-bet deficient mice, suggesting reduced type-1 cytokine secretion.[57] T-bet controls the terminal maturation of NK cells and NKT cells, and a defect in the expression of perforin and granzyme B was prominent compared with that of IFN-γ.[58] In a genital herpes simplex virus type 2 (HSV2) infection model, the cytotoxic capacity and NK-cell–mediated IFN-γ production of T-bet knockout NK cells are impaired, whereas T-bet deficiency does not impair either IFN-γ production or the cytotoxic activity of HSV2-specific CD8 T cells.[59] In the *Listeria monocytogenes* infection model, only a very mild defect in the production of IFN-γ in CD4, CD8, and NK cells was detected.[60] In a type-1 diabetes model, the number of autoaggressive CD8 lymphocytes was reduced in T-bet–deficient mice, and the production of IFN-γ was also decreased.[61] Thus, it appears clear that T-bet controls IFN-γ production in both NK and CD8 T cells, although the effects of T-bet deficiency are not obvious in some experimental models.

Reiner and colleagues[62] reported that another T-box transcription factor, Eomesodermin (Eomes), is expressed in effector CD8 and LAK cells and that the ectopic expression of Eomes in CD4 T cells induces the expression of perforin and granzyme. Dominant negative Eomes expressed in CD8 T cells inhibits both the production of IFN-γ and cytotoxic activity. A dominant function of Eomes in CD8 lineage T cells as compared with T-bet was suggested, whereas no experimental results on NK cells were reported. Future experiments addressing the relative requirements for IFN-γ production in CD4, CD8, and NK cells should increase our understanding of the nature of the tissue-specific redundant transcriptional regulation of a specific cytokine.

An ETS family transcription factor, MEF, plays critical roles in the development of NK and NKT cells.[63] MEF-deficient NK cells secrete only minimal amounts of IFN-γ, whereas MEF-deficient CD8 T cells produce IFN-γ at normal levels. In Ets-1–deficient mice, there is a significantly reduced number of NK cells and decreased cytotoxic activity against NK cell targets.[64] The effects of Ets-1 deficiency on the production of IFN-γ in NK and CD8 T cells are not known.

In CCAAT/enhancer binding protein γ (C/EBPγ)-deficient mice, the cytolytic function of splenic NK cells after stimulation with cytokines such as IL-12, IL-18, and IL-2 is significantly reduced,[65] and the production of IFN-γ in response to IL-12 and IL-18 is markedly impaired. In IRF2-deficient mice, NK cell development is impaired and the cytotoxic function of NK cells is diminished, but the effects on IFN-γ production were not analyzed.[66] Patients with mutations in the NF-κB essential modifier (NEMO) show normal percentages of peripheral blood NK cells but impaired NK cell cytotoxicity.[67] There have been no reports on the effects on IFN-γ production.

VI. NK1 AND NK2 CELL DIFFERENTIATION CULTURE IN HUMAN AND MOUSE SYSTEMS

Human NK cells at specific developmental stages have been identified based on their cell surface expression of CD161 and CD56. $CD161^+CD56^-$ immature NK cells do not express activating or inhibitory receptors, including NKp46, CD94, CD158, CD159, CD160, and CD16, and do not produce IFN-γ. This population mediates cytotoxicity through TNF-related apoptosis-inducing ligand (TRAIL) but do not show perforin/granzyme-mediated cytotoxicity.[68,69] $CD161^+$ $CD56^-$ immature NK cells become IL-13/IL-5–producing NK2 cells by cultivation with IL-4.[70] The IL-13–producing cells became NK0-like cells that produce both IL-13 and IFN-γ when cultured with IL-12 in the presence of feeder cells, and then NK0-like cells become IFN-γ single producing mature type NK1 cells. The IFN-γ single producing cell population is reported to comprise terminal differentiated mature NK cells, as evidenced by the fact that these NK cells do not give rise to NK2 cells, even in culture with IL-4, and undergo programmed cell death after activation.[70] The ability to produce IFN-γ is acquired approximately at the stage of CD56 acquisition.[15] On $CD56^{low}$ NK cells, IL-12 induces various markers that are expressed on $CD56^{high}$ mature NK cells, preventing NKp44 and CD16 expression.[71] In a series of elegant experiments involving the *in vitro* differentiation of human NK cells, Perussia and colleagues[70] demonstrated that IL-12 induces terminal differentiation of NK cells—i.e., immature $CD161^+CD56^-$ type-2 cytokine$^+$ cells mature to $CD56^+$ IFN-γ^+ cells expressing all NK markers. NK1 cells are $CD161^+CD56^+CD94^+$ and show perforin/granzyme-dependent cytotoxic activities. The expression of CD94 occurs at the stage of acquisition of IFN-γ production and is followed by the loss of IL-13 production and the induction of activating NK receptors and KIRs.

We recently established an *in vitro* murine NK1/NK2 cell differentiation system.[8] Mouse peripheral NK cells ($NK1.1^+TCR\beta^-$) separated from the spleen produce very limited amounts of IFN-γ (less than 1/10 that of NK1 cells) but no detectable levels of IL-10 or type-2 cytokines such as IL-4, IL-5, and IL-13 upon stimulation with IL-2 or PMA + ionoimycin. In our culture system, mouse NK cells are stimulated for 2 days with PMA (50 ng/mL) and Ionomycin (500 nM) in the presence of IL-2 (250 U/mL) for neutral (NK0)-culture conditions; IL-2 (250 U/mL) and IL-12 (500 U/mL) for NK1-culture conditions; and IL-2 (250 U/mL), IL-4 (500 U/mL), and anti-IFN-γ mAb for NK2-culture conditions. The resulting NK1 cells produce substantial amounts of IFN-γ and IL-10, but not IL-5 or IL-13; and NK2 cells produce IL-5 and IL-13, but not IL-4 or IFN-γ. The production of IL-5 and IL-13 were found to be STAT6 dependent. NK0, NK1, and NK2 cells all show potent cytotoxic activities against Yac-1 cells and express substantial amounts of granzyme B and perforin 1.[8]

One of the important points of this culture system is that the purified NK cells are stimulated with PMA and Ionomycin in the presence of IL-2. No feeder cells are used in the culture. We found that stimulation with PMA and Ionomycin at the initial phase of culture (2 days) was indispensable for the induction of NK1/NK2 cells, suggesting that the generation of these cells is not a phenomenon of cytokine-induced polarization of cytokine production in NK cells. In the human culture system, cultivation with γ-irradiated feeder cells or NK-sensitive tumor cells has been used with IL-2 or IL-15.[1,13,15] In these human systems, it is possible that the feeder cells or NK-sensitive tumor cells may substitute the activation of both the Ca^{2+}/calcineurin pathway and the Ras-MAPK signaling pathway. It is known that the crosslinking of CD16 on NK cells induces the activation of calcineurin and subsequent NFAT activation[72] and the activation of the ERK/MAPK cascade.[73] The requirement for the activation of both the Ca^{2+}/calcineurin pathway and the Ras-MAPK signaling pathway for NK1 and NK2 cell differentiation is reminiscent of the differentiation of helper T cells. Antigen-induced TCR-mediated signaling in naïve T cells is indispensable for the differentiation of Th1/Th2 cells.[2,74,75] This interesting analogy suggests that even in NK cells, the strength of the signaling events may influence the direction of differentiation toward NK1 and NK2 cells. In addition, NK cells express various cell surface receptor molecules that are able to transduce signals.[76] These may also control the direction of NK1/NK2 cell differentiation.

VII. BRANCHING DIFFERENTIATION (TYPE-1 VS. TYPE-2 PARADIGM) OR LINEAR DIFFERENTIATION OF NK CELLS

On the basis of a series of studies identifying human developing NK cell subsets, Loza and Perussia[77] proposed a new and interesting idea for the development of NK cells—i.e., the linear 2–0–1 development model (Fig. 1B). These authors cultured human peripheral and umbilical cord blood NK precursor cells ($CD3^-CD161^+CD56^{bright}$) with feeder cells and IL-2 + Phytohemagglutinin L (PHA-L). As described above, after cultivation with IL-12, the immature type IL-5/IL-13–producing NK2 cells differentiated first into NK0-like cells that produce both IL-13 and IFN-γ, and then into NK1 cells producing IFN-γ, which appeared to be terminally differentiated cells.[70] On the basis of these experiments, the linear sequential differentiation scenario of NK cells from type-2 immature, type-0 intermediate, to type-1 terminal differentiated NK cells was proposed. The results reported by this group reasonably support the model.[77]

On the other hand, in the mouse NK1/NK2 differentiation culture system, we detected a clear difference in the cytokine profiles of freshly prepared NK cells and NK0 cells (no production of IFN-γ, IL-10, IL-4, IL-5, or IL-13), NK1 cells (IFN-γ and IL-10 production) and NK2 cells (IL-5 and IL-13 production, but not IL-4 production).[8] These are generated from a highly purified $NK1.1^+$ population in the spleen. The results support the branching NK1/NK2 cell differentiation model (Fig. 1A), which describes Th1/Th2 cell differentiation from naïve CD4 T cells. In the mouse NK cell differentiation culture system, we have not succeeded in generating IFN-γ–producing NK1 cells from NK2 cells after second cycles of cultivation with IL-12. It is possible that the culture system is not appropriate for second cycles of type-1 and type-2 cultures; however, we have no direct evidence to suggest the linear 2–0–1 development in mouse NK cells at this time. It is also possible that the differentiation pathways of mouse and human NK cells are different. In fact, the expression pattern of various NK-specific marker molecules differ between human and mouse NK cells, and these markers are associated with the developmental and functional properties of NK cells.[78] In the mouse NK cell differentiation system, a key experiment to address this issue would be the so-called "single cell culture and analysis," in which a single $NK1.1^+$ cell is isolated and cultured in a single well to generate NK1/NK2 cells.

In the thymus, developing Vα14i NKT cells[79] express IL-4 mRNA and subsequently lose their IL-4 expression. This is reminiscent of the linear "2–0–1" differentiation discussed above for human NK cells.[77] The ontogeny of T and NKT cells takes place mostly in the thymus. Therefore, it is easy to distinguish between the differentiation (ontogeny) and functional differentiation, such as Th1/Th2 cell differentiation in the periphery. However, because there is no specific tissue for NK cell differentiation (ontogeny), both differentiation (ontogeny) and functional (NK1 and NK2) differentiation may occur at the same sites, including in the bone marrow and spleen. Thus, it is still possible that some mature NK cells in humans become IL-13– and/or IL-5–producing cells and IFN-γ–producing cells in the appropriate circumstances. We need to await the establishment of another human NK cell differentiation culture system, in which (1) cytokine nonproducing NK cells with a mature NK cell surface phenotype are separated, and (2) the activation of the MAPK cascade and Ca signals are induced at the initial phase of differentiation in addition to in the appropriate type-1 and type-2 cytokine environment. One of the key features of the linear "2–0–1" development hypothesis is that type-2 cells are not considered to be a mature functional NK cell subset. Most of the functions of NK cells have been studied with respect to immune responses to infectious microorganisms and tumors and are emphasized to be mediated by the production of IFN-γ and the cytolytic activities of NK cells. However, it remains very likely that the immunoregulatory function of type-2 cytokine-producing NK2 cells and IL-10–producing NK1 cells is exerted in other types of immune responses, including allergic reactions and autoimmune diseases. Even in the late phase of viral infections, cytokine-producing NK1/NK2 cells may play some suppressive roles against cytolytic effector NK cells or CD8 T cells. In any event, a more comprehensive investigation focused on the two differentiation models would provide a clearer view of the differentiation of functional NK1/NK2 cell differentiation.

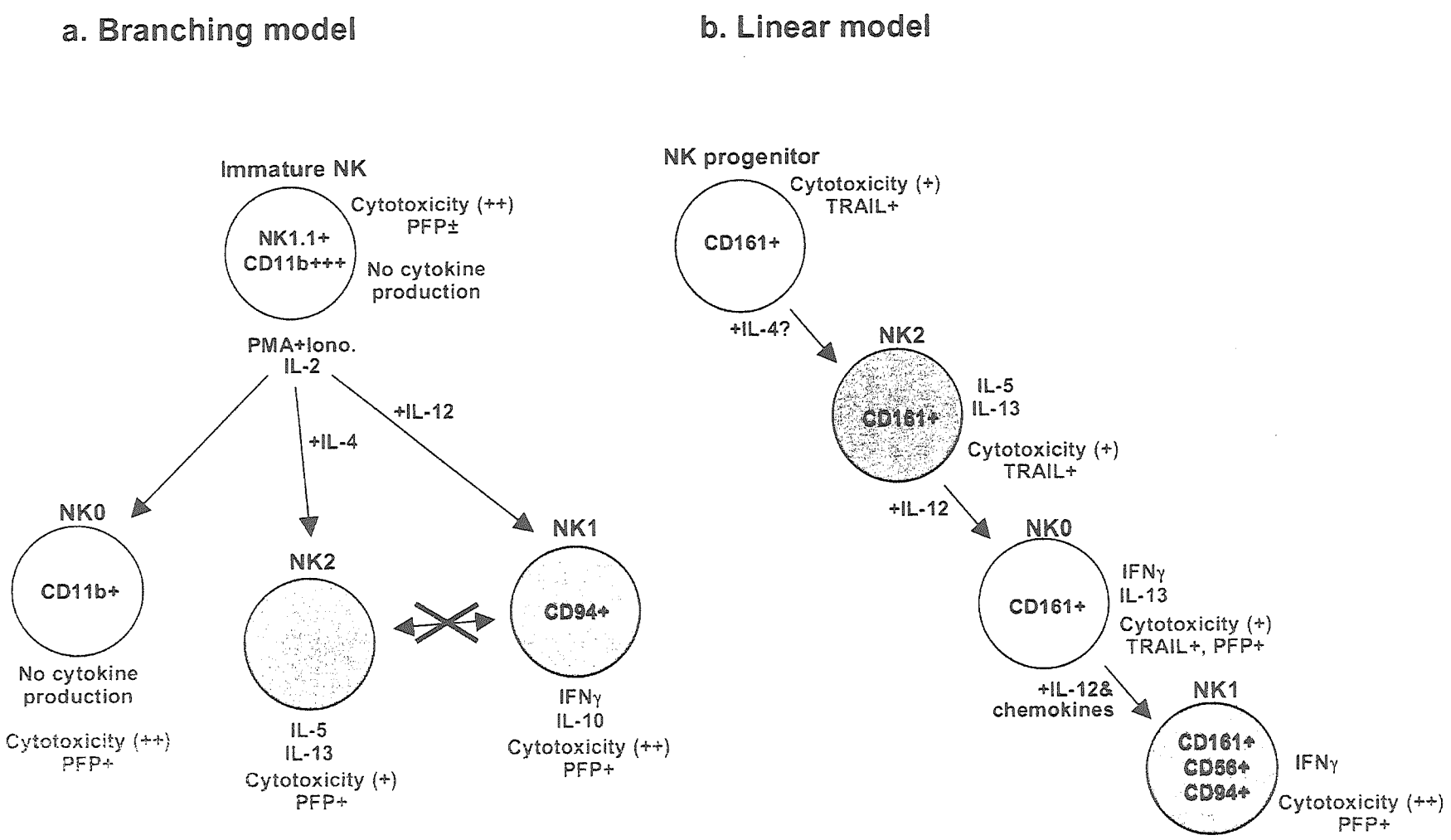

FIGURE 1. Two currently proposed NK1/NK2 cell differentiation models. **(a)** Branching type-1 vs. type-2 models. NK1 and NK2 cells differentiate from the same precursor under type-1 and type-2 culture conditions. NK0, NK1, and NK2 cells are considered to be functional mature NK cell subsets. NK1 and NK2 cells are distinct subsets, and their cytokine production profiles are stable. **(b)** Linear "2–0–1" differentiation model. NK1 cells are terminally differentiated cells that differentiate from immature NK2 cells through an intermediate NK0 phenotype in the presence of IL-12. IL-4 induces the proliferation of pre-existing IL-13 producing cells, whereas the role of IL-4 in the generation of NK2 cells is currently speculative. PFP: pore-forming protein.

VIII. PHYSIOLOGICAL ROLES OF NK2 CELLS *IN VIVO*

It has been documented that cytokine production by NK cells rather than their cytolytic activity contributes to the resistance to infectious agents.[4,5] Type-2 cytokines produced by NK2 cells may play important immunoregulatory roles in the early response to infectious microorganisms as well as T-cell responses against these pathogens.[80] Walker et al.[81] demonstrated using a peritoneal eosinophilic inflammation model, which NK cells produce IL-5 *in vivo* and which thereby have an important regulatory function in allergen-induced eosinophilic inflammation. Substantial numbers of NK cells are present in the interstitium of the normal human lung, suggesting a role for these cells in pulmonary immune responses.[82] Clinical studies have demonstrated that patients with asthma show increased numbers of NK cells accompanied by increased NK activity in the peripheral blood.[83–85] These results suggest the possible involvement of NK cells in the pathogenesis of asthma. Korsgren et al.[86] demonstrated, using eosinophilic airway inflammation in immunized and allergen-exposed mice, that NK cells play critical roles in the pathogenesis of eosinophilic airway inflammation during the process of immunization. NK cells in the peripheral blood of multiple sclerosis (MS) patients in remission are characterized by an elevation in IL-5 mRNA expression and production, decreased IL-12Rβ2 mRNA, and a higher expression of CD95.[53] These NK2-like NK cells were not detected when there was a relapse of the MS. Thus, NK2-like cells appear to control the remission of MS. Recently, increased numbers of NK2 cells in asthmatic patients have also been reported.[87] In atopic dermatitis patients, freshly isolated NK cells release higher amounts of type-2 cytokines and IFN-γ, as compared with NK cells from healthy individuals.[52] It is interesting that NKG2A expression is lower in NK2 cells compared with freshly prepared NK cells or NK1 cells in healthy individuals, but is the same in atopic dermatitis patients. No functional Ig isotype switching to IgE was observed in the system in which differentiated NK2 cells are co-cultivated with purified B cells, although NK1 cells show a significant anti-IgE effect.[52] These results suggest some association between the presence of NK2 cells and certain allergic and autoimmune disorders, but direct evidence of the causal effects of NK2 cells in the pathogenesis of these diseases is still needed. The reports are summarized in Table 1.

IX. CONCLUSIONS

Accumulating evidence indicating the presence of type-2 cytokine-producing NK2 cells supports the notion that NK2 cells constitute a distinct functional subset of NK cells. NK2 cells produce IL-5 and IL-13, but not IL-4 or IL-10, and this unique cytokine production profile suggests a specific immunoregulatory function of NK2 cells under some physiological and/or pathological conditions. Further analyses are still needed to address which transcription factors are critical for NK2 cell differentiation and how various transcription factors cooperate or counteract each other to generate NK1/NK2 cell subsets. Research efforts aimed at the manipulation of NK1/NK2 cell differentiation may be helpful for establishing novel approaches to the treatment of allergic and autoimmune diseases, as well as infectious diseases and tumors.

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REFERENCES

1. Peritt D, Robertson S, Gri G, Showe L, Aste-Amezaga M, Trinchieri G. Differentiation of human NK cells into NK1 and NK2 subsets. J Immunol. 1998;161 (11):5821–4.
2. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature. 1996;383 (6603):787–93.
3. O'Garra A. Immunology. Commit ye helpers. Nature. 2000;404(6779):719–20.
4. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol. 1999;17:189–220.
5. Scharton-Kersten TM, Sher A. Role of natural killer cells in innate resistance to protozoan infections. Curr Opin Immunol. 1997;9(1):44–51.
6. Miller JS. The biology of natural killer cells in cancer, infection, and pregnancy. Exp Hematol. 2001;29(10): 1157–68.
7. Smyth MJ, Hayakawa Y, Takeda K, Yagita H. New aspects of natural-killer-cell surveillance and therapy of cancer. Nat Rev Cancer. 2002;2(11):850–61.
8. Katsumoto T, Kimura M, Yamashita M, Hosokawa H, Hashimoto K, Hasegawa A, Omori M, Miyamoto T, Taniguchi M, Nakayama T. STAT6-dependent differentiation and production of IL-5 and IL-13 in murine NK2 cells. J Immunol. 2004;173(8):4967–75.
9. Anegon I, Cuturi MC, Trinchieri G, Perussia B. Interaction of Fc receptor (CD16) ligands induces transcription of interleukin 2 receptor (CD25) and lymphokine genes and expression of their products in human natural killer cells. J Exp Med. 1988;167(2): 452–72.
10. Cassatella MA, Anegon I, Cuturi MC, Griskey P, Trinchieri G, Perussia B. FcγR(CD16) interaction with ligand induces Ca^{2+} mobilization and phosphoinositide turnover in human natural killer cells. Role of Ca^{2+} in FcγR(CD16)-induced transcription and expression of lymphokine genes. J Exp Med. 1989;169 (2):549–67.
11. Cuturi MC, Anegon I, Sherman F, Loudon R, Clark SC, Perussia B, Trinchieri G. Production of hematopoietic colony-stimulating factors by human natural killer cells. J Exp Med. 1989;169(2):569–83.
12. Warren HS, Kinnear BF, Phillips JH, Lanier LL. Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10, and IL-12. J Immunol. 1995;154(10):5144–52.
13. Deniz G, Akdis M, Aktas E, Blaser K, Akdis CA. Human NK1 and NK2 subsets determined by purification of IFN-γ-secreting and IFN-γ-nonsecreting NK cells. Eur J Immunol. 2002;32(3):879–84.
14. Hoshino T, Winkler-Pickett RT, Mason AT, Ortaldo JR, Young HA. IL-13 production by NK cells: IL-13-producing NK and T cells are present in vivo in the absence of IFN-γ. J Immunol. 1999;162(1):51–9.
15. Loza MJ, Zamai L, Azzoni L, Rosati E, Perussia B. Expression of type 1 (interferon gamma) and type 2 (interleukin-13, interleukin-5) cytokines at distinct stages of natural killer cell differentiation from progenitor cells. Blood. 2002;99(4):1273–81.
16. Loza MJ, Peters SP, Zangrilli JG, Perussia B. Distinction between IL-13$^+$ and IFN-γ$^+$ natural killer cells and regulation of their pool size by IL-4. Eur J Immunol. 2002;32(2):413–23.
17. Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J Biol Chem. 1997;272(34):21597–603.
18. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. 1997;89(4):587–96.
19. Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, Murphy KM. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. Immunity. 1998; 9(5):745–55.
20. Yamashita M, Ukai-Tadenuma M, Kimura M, Omori M, Inami M, Taniguchi M, Nakayama T. Identification of a conserved GATA3 response element upstream proximal from the interleukin-13 gene locus. J Biol Chem. 2002;277(44):42399-408.
21. Omori M, Yamashita M, Inami M, Ukai-Tadenuma M, Kimura M, Nigo Y, Hosokawa H, Hasegawa A, Taniguchi M, Nakayama T. CD8 T cell-specific downregulation of histone hyperacetylation and gene activation of the IL-4 gene locus by ROG, repressor of GATA. Immunity. 2003;19(2):281–94.
22. Inami M, Yamashita M, Tenda Y, Hasegawa A, Kimura M, Hashimoto K, Seki N, Taniguchi M, Nakayama T. CD28 costimulation controls histone hyperacetylation of the interleukin 5 gene locus in developing th2 cells. J Biol Chem. 2004;279(22): 23123–33.
23. Kishikawa H, Sun J, Choi A, Miaw SC, Ho IC. The cell type-specific expression of the murine IL-13 gene is regulated by GATA-3. J Immunol. 2001;167(8): 4414–20.
24. Lavenu-Bombled C, Trainor CD, Makeh I, Romeo PH, Max-Audit I. Interleukin-13 gene expression is regulated by GATA-3 in T cells: role of a critical association of a GATA and two GATG motifs. J Biol Chem. 2002;277(21):18313–21.
25. Miaw SC, Choi A, Yu E, Kishikawa H, Ho IC. ROG, repressor of GATA, regulates the expression of cytokine genes. Immunity. 2000;12(3):323–33.
26. Yu CR, Kirken RA, Malabarba MG, Young HA, Ortaldo JR. Differential regulation of the Janus kinase-

STAT pathway and biologic function of IL-13 in primary human NK and T cells: a comparative study with IL-4. J Immunol. 1998;161(1):218–27.

27. Samson SI, Richard O, Tavian M, Ranson T, Vosshenrich CA, Colucci F, Buer J, Grosveld F, Godin I, Di Santo JP. GATA-3 promotes maturation, IFN-γ production, and liver-specific homing of NK cells. Immunity. 2003;19(5):701–11.
28. Chakir H, Camilucci AA, Filion LG, Webb JR. Differentiation of murine NK cells into distinct subsets based on variable expression of the IL-12Rβ2 subunit. J Immunol. 2000;165(9):4985–93.
29. Mehrotra PT, Donnelly RP, Wong S, Kanegane H, Geremew A, Mostowski HS, Furuke K, Siegel JP, Bloom ET. Production of IL-10 by human natural killer cells stimulated with IL-2 and/or IL-12. J Immunol. 1998;160(6):2637–44.
30. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683–765.
31. Chan SH, Perussia B, Gupta JW, Kobayashi M, Pospisil M, Young HA, Wolf SF, Young D, Clark SC, Trinchieri G. Induction of interferon γ production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. J Exp Med. 1991;173(4):869–79.
32. Son YI, Dallal RM, Mailliard RB, Egawa S, Jonak ZL, Lotze MT. Interleukin-18 (IL-18) synergizes with IL-2 to enhance cytotoxicity, interferon-γ production, and expansion of natural killer cells. Cancer Res. 2001;61(3):884–8.
33. Hoshino T, Wiltrout RH, Young HA. IL-18 is a potent coinducer of IL-13 in NK and T cells: a new potential role for IL-18 in modulating the immune response. J Immunol. 1999;162(9):5070–7.
34. Sareneva T, Julkunen I, Matikainen S. IFN-α and IL-12 induce IL-18 receptor gene expression in human NK and T cells. J Immunol. 2000;165(4):1933–8.
35. Nakahira M, Ahn HJ, Park WR, Gao P, Tomura M, Park CS, Hamaoka T, Ohta T, Kurimoto M, Fujiwara H. Synergy of IL-12 and IL-18 for IFN-γ gene expression: IL-12-induced STAT4 contributes to IFN-γ promoter activation by up-regulating the binding activity of IL-18-induced activator protein 1. J Immunol. 2002;168(3):1146–53.
36. Matikainen S, Paananen A, Miettinen M, Kurimoto M, Timonen T, Julkunen I, Sareneva T. IFN-α and IL-18 synergistically enhance IFN-γ production in human NK cells: differential regulation of Stat4 activation and IFN-γ gene expression by IFN-α and IL-12. Eur J Immunol. 2001;31(7):2236–45.
37. Minty A, Chalon P, Derocq JM, Dumont X, Guillemot JC, Kaghad M, Labit C, Leplatois P, Liauzun P, Miloux B, Mynty C, Casellas P, Loison G, Lupker J, Shire D, Ferrara P, Caput D. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. Nature. 1993;362(6417):248–50.
38. Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today. 1994; 15(1):19–26.
39. Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, Akita K, Namba M, Tanabe F, Konishi K, Fukuda S, Kurimoto M. Cloning of a new cytokine that induces IFN-γ production by T cells. Nature. 1995;378(6552):88–91.
40. Chakir H, Lemay AM, Webb JR. Cytokine expression by murine DX5+ cells in response to IL-12, IL-18, or the combination of IL-12 and IL-18. Cell Immunol. 2001;212(1):71–81.
41. Lauwerys BR, Garot N, Renauld JC, Houssiau FA. Cytokine production and killer activity of NK/T-NK cells derived with IL-2, IL-15, or the combination of IL-12 and IL-18. J Immunol. 2000;165(4):1847–53.
42. Colucci F, Caligiuri MA, Di Santo JP. What does it take to make a natural killer? Nat Rev Immunol. 2003;3(5):413–25.
43. Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. J Immunol. 1986;136(12):4480–6.
44. Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, Carson WE, Caligiuri MA. Human natural killer cells: a unique innate immunoregulatory role for the CD56^{bright} subset. Blood. 2001;97(10):3146–51.
45. Nagler A, Lanier LL, Cwirla S, Phillips JH. Comparative studies of human FcRIII-positive and negative natural killer cells. J Immunol. 1989;143(10): 3183–91.
46. Ellis TM, Fisher RI. Functional heterogeneity of Leu 19$^{"bright"+}$ and Leu 19$^{"dim"+}$ lymphokine-activated killer cells. J Immunol. 1989;142(8):2949–54.
47. Robertson MJ, Soiffer RJ, Wolf SF, Manley TJ, Donahue C, Young D, Herrmann SH, Ritz J. Response of human natural killer (NK) cells to NK cell stimulatory factor (NKSF): cytolytic activity and proliferation of NK cells are differentially regulated by NKSF. J Exp Med. 1992;175(3):779–88.
48. Arase H, Saito T, Phillips JH, Lanier LL. Cutting edge: the mouse NK cell-associated antigen recognized by DX5 monoclonal antibody is CD49b (alpha 2 integrin, very late antigen-2). J Immunol. 2001;167(3): 1141–4.
49. Frey M, Packianathan NB, Fehniger TA, Ross ME, Wang WC, Stewart CC, Caligiuri MA, Evans SS. Differential expression and function of L-selectin on CD56^{bright} and CD56^{dim} natural killer cell subsets. J Immunol. 1998;161(1):400–8.

50. Fehniger TA, Cooper MA, Nuovo GJ, Cella M, Facchetti F, Colonna M, Caligiuri MA. CD56^{bright} natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. Blood. 2003;101(8):3052–7.

51. Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol. 2002;2(9):656–63.

52. Aktas E, Akdis M, Bilgic S, Disch R, Falk CS, Blaser K, Akdis C, Deniz G. Different natural killer (NK) receptor expression and immunoglobulin E (IgE) regulation by NK1 and NK2 cells. Clin Exp Immunol. 2005;140(2):301–9.

53. Takahashi K, Miyake S, Kondo T, Terao K, Hatakenaka M, Hashimoto S, Yamamura T. Natural killer type 2 bias in remission of multiple sclerosis. J Clin Invest. 2001;107(5):R23–9.

54. Glimcher LH, Townsend MJ, Sullivan BM, Lord GM. Recent developments in the transcriptional regulation of cytolytic effector cells. Nat Rev Immunol. 2004;4(11):900–11.

55. Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in TH1 lineage commitment and IFN-γ production in CD4 and CD8 T cells. Science. 2002;295(5553): 338–42.

56. Sullivan BM, Juedes A, Szabo SJ, von Herrath M, Glimcher LH. Antigen-driven effector CD8 T cell function regulated by T-bet. Proc Natl Acad Sci U S A. 2003;100(26):15818–23.

57. Finotto S, Neurath MF, Glickman JN, Qin S, Lehr HA, Green FH, Ackerman K, Haley K, Galle PR, Szabo SJ, Drazen JM, De Sanctis GT, Glimcher LH. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. Science. 2002;295(5553):336–8.

58. Townsend MJ, Weinmann AS, Matsuda JL, Salomon R, Farnham PJ, Biron CA, Gapin L, Glimcher LH. T-bet regulates the terminal maturation and homeostasis of NK and Vα14*i* NKT cells. Immunity. 2004;20(4):477–94.

59. Svensson A, Nordstrom I, Sun JB, Eriksson K. Protective immunity to genital herpes simpex virus type 2 infection is mediated by T-bet. J Immunol. 2005; 174(10):6266–73.

60. Way SS, Wilson CB. Cutting edge: immunity and IFN-γ production during Listeria monocytogenes infection in the absence of T-bet. J Immunol. 2004; 173(10):5918–22.

61. Juedes AE, Rodrigo E, Togher L, Glimcher LH, von Herrath MG. T-bet controls autoaggressive CD8 lymphocyte responses in type 1 diabetes. J Exp Med. 2004;199(8):1153–62.

62. Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, Zediak VP, Banica M, DiCioccio CB, Gross DA, Mao CA, Shen H, Cereb N, Yang SY, Lindsten T, Rossant J, Hunter CA, Reiner SL. Control of effector CD8$^+$ T cell function by the transcription factor *Eomesodermin*. Science. 2003;302(5647): 1041–3.

63. Lacorazza HD, Miyazaki Y, Di Cristofano A, Deblasio A, Hedvat C, Zhang J, Cordon-Cardo C, Mao S, Pandolfi PP, Nimer SD. The ETS protein MEF plays a critical role in perforin gene expression and the development of natural killer and NK-T cells. Immunity. 2002;17(4):437–49.

64. Barton K, Muthusamy N, Fischer C, Ting CN, Walunas TL, Lanier LL, Leiden JM. The Ets-1 transcription factor is required for the development of natural killer cells in mice. Immunity. 1998;9(4):555–63.

65. Kaisho T, Tsutsui H, Tanaka T, Tsujimura T, Takeda K, Kawai T, Yoshida N, Nakanishi K, Akira S. Impairment of natural killer cytotoxic activity and interferon gamma production in CCAAT/enhancer binding protein gamma-deficient mice. J Exp Med. 1999; 190(11):1573–82.

66. Lohoff M, Duncan GS, Ferrick D, Mittrucker HW, Bischof S, Prechtl S, Rollinghoff M, Schmitt E, Pahl A, Mak TW. Deficiency in the transcription factor interferon regulatory factor (IRF)-2 leads to severely compromised development of natural killer and T helper type 1 cells. J Exp Med. 2000;192(3): 325–36.

67. Orange JS, Brodeur SR, Jain A, Bonilla FA, Schneider LC, Kretschmer R, Nurko S, Rasmussen WL, Kohler JR, Gellis SE, Ferguson BM, Strominger JL, Zonana J, Ramesh N, Ballas ZK, Geha RS. Deficient natural killer cell cytotoxicity in patients with IKK-gamma/NEMO mutations. J Clin Invest. 2002;109(11): 1501–9.

68. Bennett IM, Zatsepina O, Zamai L, Azzoni L, Mikheeva T, Perussia B. Definition of a natural killer NKR-P1A$^+$/CD56$^-$/CD16$^-$ functionally immature human NK cell subset that differentiates in vitro in the presence of interleukin 12. J Exp Med. 1996; 184(5):1845–56.

69. Zamai L, Ahmad M, Bennett IM, Azzoni L, Alnemri ES, Perussia B. Natural killer (NK) cell-mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells. J Exp Med. 1998;188(12):2375–80.

70. Loza MJ, Perussia B. Final steps of natural killer cell maturation: a model for type 1-type 2 differentiation? Nat Immunol. 2001;2(10):917–24.

71. Loza MJ, Perussia B. The IL-12 signature: NK cell terminal CD56^{+high} stage and effector functions. J Immunol. 2004;172(1):88–96.

72. Aramburu J, Azzoni L, Rao A, Perussia B. Activation and expression of the nuclear factors of activated T cells, NFATp and NFATc, in human natural killer cells: regulation upon CD16 ligand binding. J Exp Med. 1995;182(3):801–10.

73. Trotta R, Puorro KA, Paroli M, Azzoni L, Abebe B, Eisenlohr LC, Perussia B. Dependence of both spontaneous and antibody-dependent, granule exocytosis-mediated NK cell cytotoxicity on extracellular signal-regulated kinases. J Immunol. 1998;161(12): 6648–56.
74. Yamashita M, Kimura M, Kubo M, Shimizu C, Tada T, Perlmutter RM, Nakayama T. T cell antigen receptor-mediated activation of the Ras/mitogen-activated protein kinase pathway controls interleukin 4 receptor function and type-2 helper T cell differentiation. Proc Natl Acad Sci U S A. 1999;96(3):1024–9.
75. Yamashita M, Katsumata M, Iwashima M, Kimura M, Shimizu C, Kamata T, Shin T, Seki N, Suzuki S, Taniguchi M, Nakayama T. T cell receptor-induced calcineurin activation regulates T helper type 2 cell development by modifying the interleukin 4 receptor signaling complex. J Exp Med. 2000;191(11):1869–79.
76. Lanier LL. Natural killer cell receptor signaling. Curr Opin Immunol. 2003;15(3):308–14.
77. Perussia B, Loza MJ. Linear "2-0-1" lymphocyte development: hypotheses on cellular bases for immunity. Trends Immunol. 2003;24(5):235–41.
78. Lanier LL. NK cell receptors. Annu Rev Immunol. 1998;16:359–93.
79. Benlagha K, Kyin T, Beavis A, Teyton L, Bendelac A. A thymic precursor to the NK T cell lineage. Science. 2002;296(5567):553–5.
80. Trinchieri G. Natural killer cells wear different hats: effector cells of innate resistance and regulatory cells of adaptive immunity and of hematopoiesis. Semin Immunol. 1995;7(2):83–8.
81. Walker C, Checkel J, Cammisuli S, Leibson PJ, Gleich GJ. IL-5 production by NK cells contributes to eosinophil infiltration in a mouse model of allergic inflammation. J Immunol. 1998;161(4):1962–9.
82. Weissler JC, Nicod LP, Lipscomb MF, Toews GB. Natural killer cell function in human lung is compartmentalized. Am Rev Respir Dis. 1987;135(4):941–9.
83. Timonen T, Stenius-Aarniala B. Natural killer cell activity in asthma. Clin Exp Immunol. 1985;59(1): 85–90.
84. Jira M, Antosova E, Vondra V, Strejcek J, Mazakova H, Prazakova J. Natural killer and interleukin-2 induced cytotoxicity in asthmatics. I. Effect of acute antigen-specific challenge. Allergy. 1988;43(4): 294–8.
85. Krejsek J, Kral B, Vokurkova D, Derner V, Touskova M, Parakova Z, Kopecky O. Decreased peripheral blood γδ T cells in patients with bronchial asthma. Allergy. 1998;53(1):73–7.
86. Korsgren M, Persson CG, Sundler F, Bjerke T, Hansson T, Chambers BJ, Hong S, Van Kaer L, Ljunggren HG, Korsgren O. Natural killer cells determine development of allergen-induced eosinophilic airway inflammation in mice. J Exp Med. 1999;189(3): 553–62.
87. Wei H, Zhang J, Xiao W, Feng J, Sun R, Tian Z. Involvement of human natural killer cells in asthma pathogenesis: natural killer 2 cells in type 2 cytokine predominance. J Allergy Clin Immunol. 2005;115(4): 841–7.
88. Wingett D, Nielson CP. Divergence in NK cell and cyclic AMP regulation of T cell CD40L expression in asthmatic subjects. J Leukoc Biol. 2003;74(4): 531–41.

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CASE REPORT

Sublingual Immunotherapy for Japanese Cedar Pollinosis

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ABSTRACT

Background: Although subcutaneous immunotherapy may cure allergic diseases, it is not commonly used in Japan because of the pain and risk of anaphylactic shock. Sublingual immunotherapy(SLIT)overcomes these limitations and although it is the most advanced form of local immunotherapy for clinical application, it is not used in Japan nor has it been extensively studied.

Methods: After obtaining approval from the Ethics Committee of Nippon Medical School and informed consent from five patients with cedar pollinosis(one man, four women ; age range, 38–66 years), administration of a therapeutic extract was started in July 2001 or later(mean treatment period, 13.4 months). The clinical efficacy of SLIT and its influence on the quality of life, as measured by the Japanese Allergic Rhinitis QOL Standard Questionnaire, and the incidence of side effects were evaluated in 2003.

Results: Between February and April the mean severity score was 1.44 in the patients undergoing SLIT and 1.86 in the patients undergoing pharmacotherapy, and the respective mean QOL total scores during the season were 3.82 and 10.0. Neither systemic nor local side effects occurred during SLIT.

Conclusions: SLIT is safe and effective for Japanese cedar pollinosis.

KEY WORDS

allergic rhinitis, Japanese Allergic Rhinitis QOL Standard Questionnaire(JRQLQ), Japanese cedar pollinosis, quality of life, sublingual immunotherapy(SLIT)

INTRODUCTION

Subcutaneous injection immunotherapy is a painful procedure and has the risk of anaphylactic shock as a side effect, which is why it is not commonly used in Japan. To overcome these limitations, patients in Europe and the United States can undergo local immunotherapy in which the antigen is administered to the nasal, intestinal or tracheal mucosa, and of these, sublingual immunotherapy (SLIT) is the most advanced clinical application. Placebo-control studies of SLIT against house dust,[1-3] grass,[4-7] weeds[8] and *Parietaria* [9,10] have demonstrated a marked improvement in clinical symptoms after immunotherapy compared with placebo, and a significantly lower incidence of side effects than with injection immunotherapy. In Japan, immunotherapy consists of subcutaneous injection only and local immunotherapy is not used in clinical practice. Other than our pilot study,[11] SLIT has not been investigated in Japan. In the present study conducted in 2003 we evaluated the clinical efficacy of SLIT, its influence on the quality of life (QOL) and the incidence of side effects in patients with cedar pollinosis.

CLINICAL SUMMARY

SUBJECTS

After the protocol was approved by the Ethics Committee of Nippon Medical School and informed consent was given by five patients with cedar pollinosis (one man, four women ; age range, 38–66 years(Table 1)), administration of a therapeutic extract was started.

The main antigen was cedar and none of the patients had other allergic diseases or double sensitization with other antigens that would influence the evaluation of the treatment response during the cedar pollen season. Treatment was started in July 2001 or later, and clinical efficacy was evaluated in April 2003 (mean treatment period, 13.4 months).

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Table 1 Profile of patients

	SLIT	Pharmacotherapy
Age (mean)	47.3	45.0
Sex		
female	4	4
male	1	1
Duration of SLIT (mean)	13.4 months	
Severity		
Mild	0	0
Moderate	2	4
Severe	3	1

Table 2 Schedule of sublingual administration

	1st week 1 : 50000	2nd week 1 : 5000	3rd week 1 : 500	4th week 1 : 500
1st day	1 drop	1 drop	1 drop	20 drops
2nd day	2 drops	2 drops	2 drops	
3rd day	3 drops	3 drops	4 drops	
4th day	4 drops	4 drops	8 drops	
5th day	6 drops	6 drops	12 drops	20 drops
6th day	8 drops	8 drops	16 drops	
7th day	10 drops	10 drops	20 drops	

The pharmacotherapy group consisted of five patients with cedar pollinosis who consulted the outpatient clinic of the Department of Otorhinolaryngology at Nippon Medical School Hospital during the same period (one man, four women ; age range, 36–53 years (Table 1)).

METHODS

Japanese cedar antigen extract (1 : 20) (Hollister-Stier Laboratories LLC, Spokane, WA, USA) was diluted prior to use, but because it is not standardized, there are no data about its major allergen content. In our preliminary study, the concentration of the major Japanese cedar pollen allergen, Cry j 1, was regarded as being 7.7–16.5 μg/ml.[12] Crumbs containing the antigen extract were placed under the tongue for approximately 2 min and then spat out ('sublingual spit-out'). The subjects attended the outpatient clinic, weekly from week 1 to week 3 and then fortnightly from week 4 of treatment, where they obtained the therapeutic extract and administered it at home in increasing doses (Table 2).

Clinical Symptoms(Nasal Symptom Score)

Nasal allergic symptoms were evaluated from patient diaries and symptom/severity scores were calculated according to the Japanese Practice Guideline for Allergic Rhinitis (4th edition).[13] The most severe status was scored as 4, severe status as 3, moderate status as 2, and mild status as 1 (Table 3).

Medication Score

In the drug therapy group, the various medications were also scored according to the guideline[13] as follows : first- or second-generation antihistamines and mast cell stabilizers, 1 point ; topical steroids, 2 points ; vasoconstrictor or anticholinergic nasal drop preparations, 1 point ; antihistaminic eye drop preparations, 1 point ; steroid eye spray preparations, 2 points ; the period during which the dose is increased, 0.5 points ; the maintenance dose, 1 point ; and mixed preparation of an antihistaminic agent and betamethasone, 3 points (Table 4).

Evaluation of QOL

We evaluated changes in the subjects' QOL during the cedar pollen season using the Japanese Allergic Rhinitis QOL Standard Questionnaire (JRQLQ ; 2002),[14] which has three parts : (I) nasal/ocular symptoms, (II) 17 questions about QOL and (III) a comprehensive evaluation (face scale).

The QOL questions investigated issues in six domains ('daily life', 'outdoor life', 'social life', 'sleep', 'fatigue' and 'emotion'), such as 'interference with study, work, or housework', 'lack of concentration', 'decline in thinking power', 'inconvenience with reading and newspapers', 'debilitating memory loss', 'interference with outdoor activities such as sports, picnic, etc', 'limitation on going out', 'interference with social activities', 'interference with conversation/telephone conversation', 'embarrassment from presumed public attention', 'sleep disorder', 'feeling of weariness', 'fatigue', 'nervousness', 'frustrated', 'gloominess' and 'lack of satisfaction with daily life'. Responses were evaluated using five grades.

PATHOLOGICAL FINDINGS

In 2003, the amount of cedar pollen in Chiyoda-ku, central Tokyo, was 3,622 grains/cm^2, which was similar to the annual average (according to a survey conducted by the Bureau of Public Health Tokyo Metropolitan Government).

CHANGES IN CLINICAL SYMPTOMS (NASAL SYMPTOM SCORE)

As shown in Table 5 the mean symptom scores in the SLIT group for sneezing, nasal discharge, nasal obstruction, and ocular symptoms between February and April were 1.07, 1.30, 0.56, and 0.39, respectively. All scores were highest in March and rapidly returned to the February values in April. The respective mean symptom scores in the pharmacotherapy group were 1.07, 1.76, 1.01, and 0.80 (Table 5). All scores were highest in March, as in the SLIT group, but in April there was a prolonged interval until symptoms were relieved.

The mean severity scores between February and April were 1.44 in the SLIT group and 1.86 in the pharmacotherapy group (Table 6).

Table 3 Criteria for symptom score and severity score

Grade	No. of sneezing attacks per day	No. of nose blows per day	Nasal obstruction
Most severe (4 points)	>20	>20	Complete (all day)
Severe (3 points)	11–20	11–20	Severe (considerable amount of mouth breathing required)
Moderate (2 points)	6–10	6–10	Marked (frequent mouth breathing)
Mild (1 point)	1–5	1–5	Present (no mouth breathing)
No symptoms (0 point)	0	0	None

Table 4 Criteria for medication score

1st, 2nd generation anti-histamines, mast cell stabilizers	1 point
Topical steroids	2 points
Decongestant, anti-cholinergic agents	1 point
Ocular anti-histamines	1 point
Ocular steroids	2 points
Specific immunotherapy	
During step up	0.5 points
During maintenance dose	1 point
Oral steroids and anti-histamines	3 points

CHANGES IN THE MEDICATION SCORE

The mean medication scores between February and April were 0.21 in the SLIT group and 1.85 in the pharmacotherapy group(Table 7).

CHANGES IN THE QOL

The mean QOL total scores during the pollen season were 3.82 in the SLIT group and 10.0 in the drug therapy group(Table 8).

SIDE EFFECTS

Neither systemic nor local side effects occurred during SLIT.

DISCUSSION

The mechanism of action for SLIT, or for conventional allergen immunotherapy, is still unclear, but for allergen-specific immunotherapy, reduction of effector cells[15,16] and blocking antibody[17-20] have been the conventional theories. Recently, however, it has become widely accepted that immunotherapy may modify the T cell response to natural allergens because of T cell anergy and/or immune deviation.[21-24] For SLIT in particular, allergen administered to the oral mucosa accumulates in the submandibular lymph node, in which the immune response occurs[25] and peaks at approximately 2 h after administraton.[26] Of the local immunotherapy modalities, SLIT is the most effective with a lower incidence of side effects, which complies with the WHO position paper on allergen immunotherapy requiring a new route of administration, such as local immunotherapy, and treatment that does not cause anaphylaxis, such as peptide therapy.[27] However, only subcutaneous immunotherapy is used for Japanese cedar pollinosis and other than our pilot study,[11] and the present report, SLIT is an unknown treatment.

Approximately 13% of the Japanese population are affected by Japanese cedar pollinosis[28] and the proportion of severe status patients is higher than with grass or ragweed pollinosis, which are the representative conditions in other countries, and the symptoms persist for about 3 months, becoming a social issue. When the amount of pollen increases, patients show more severe symptoms, and the number of severe status patients is greatest in mid-March(late season)when the pollen count reaches its peak. Substantial antigen exposure enhances the antigen-antibody reaction in the airways (airway hypersensitivity), which is the mechanism involved in severe pollinosis, and immunotherapy with antigen-specific effects may control the exacerbation of the symptoms in the latter half of the cedar pollen season by inhibiting antigen-related enhancement of nasal mucosal hypersensitivity. In the present study, SLIT both inhibited the exacerbation of symptoms in the latter half of the season and reduced their severity throughout the season. Furthermore, there were neither local nor systemic side effects, as reported elsewhere for other antigens.

SLIT for cedar pollinosis is a new therapy and in the future SLIT may by indicated for patients with nasal allergy caused by other allergens such as house dust mites or animal dander through improvement of the administration schedule and establishing the dose at which the most potent effects are achieved. Therefore, a multicenter study involving a large number of patients should be conducted.

ACKNOWLEDGEMENTS

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Table 5 Monthly mean change in symptom score

		Feb.	Mar.	Apr.	Mean of 3 months
Score of sneezing	Pharmacotherapy	0.44	1.35	1.36	1.07
	SLIT	0.84	1.48	0.87	1.07
Score of nasal discharge	Pharmacotherapy	1.02	2.19	2.00	1.76
	SLIT	0.91	1.79	1.16	1.30
Score of nasal obstruction	Pharmacotherapy	0.48	1.37	1.15	1.01
	SLIT	0.31	0.86	0.49	0.56
Eye symptom score	Pharmacotherapy	0.46	1.14	0.76	0.80
	SLIT	0.26	0.68	0.21	0.39

Table 6 Monthly mean change in severity score

		Feb.	Mar.	Apr.	Mean of 3 months
Severity score	Pharmacotherapy	1.14	2.25	2.13	1.86
	SLIT	1.11	1.92	1.26	1.44

Table 7 Monthly mean change in medication score

		Feb.	Mar.	Apr.	Mean of 3 months
Medication score	Pharmacotherapy	1.49	1.90	2.13	1.85
	SLIT	0.07	0.43	0.12	0.21

Table 8 Monthly mean change in QOL score

		Feb.	Mar.	Apr.	Mean of 3 months
QOL score	Pharmacotherapy	6.0	16.8	7.2	10.0
	SLIT	1.67	5.8	3.82	3.82

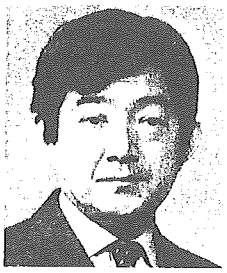
REFERENCES

1. Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite : a double-blind study. *Allergol. Immunopathol. (Madr.)* 1990;**18**:277-284.
2. Mungan D, Misirligil Z, Gurbuz L. Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma : a placebo controlled study. *Ann. Allergy Asthma Immunol.* 1999;**82**:485-490.
3. Guez S, Vatrinet C, Fadel R, Andre C. House-dust-mite sublingual-swallow immunotherapy (SLIT) in perennial rhinitis : a double-blind, placebo-controlled study. *Allergy* 2000;**55**:369-375.
4. Di Rienzo V, Puccinelli P, Frati F, Parmiani S. Grass pollen specific sublingual/ swallow immunotherapy in children : open-controlled comparison among different treatment protocols. *Allergol. Immunopathol. (Madr.)* 1999;**27**: 145-151.
5. Hordijk GJ, Antvelink JB, Luwema RA. Sublingual immunotherapy with a standardised grass pollen extract : a double-blind placebo-controlled study. *Allergol. Immunopathol. (Madr.)* 1998;**26**:234-240.
6. Clavel R, Bousquet J, Andre C. Clinical efficacy of sublingual-swallow immunotherapy : a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis. *Allergy* 1998;**53**:493-498.
7. Pradalier A, Basset D, Claudel A *et al.* Sublingual-swallow immunotherapy (SLIT) with a standardized five-grass-pollen extract (drops and sublingual tablets) versus placebo in seasonal rhinitis. *Allergy* 1999;**54**:819-828.
8. Valle C, Bazzi S, Berra D, Sillano V, Puccinelli P, Parmiani S. Effects of sublingual immunotherapy in patients sensitised to Ambrosia : an open controlled study. *Allergol. Immunopathol. (Madr.)* 2000;**28**:311-317.
9. Troise C, Voltolini S, Canessa A, Pecora S, Negrini AC. Sublingual immunotherapy in *Parietaria* pollen-induced rhinitis : a double-blind study. *J. Invest. Allergol. Clin. Immunol.* 1995;**5**:25-30.
10. Purello-D'Ambrosio F, Gangemi S, Isola S *et al.* Sublingual immunotherapy : a double-blind, placebo-controlled trial with *Parietaria judaica* extract standardized in mass units in patients with rhinoconjunctivitis, asthma, or both. *Allergy* 1999;**54**:968-973.
11. Okubo K, Gotoh M, Shimada K, Okuda M, Yagi T, Dairiki K. Sublingual immuno-therapy for Japanese cedar pollino-

sis : pilot study. *Jpn. J. Rhinol.* 2002;**41**:30-35(in Japanese).

12. Okubo K, Takizawa R, Gotoh M, Okuda M. Experience of specific immunotherapy with standardized Japanese cedar pollen extract. *Arerugi* 2001;**50**:520-527(in Japanese).
13. *Japanese Practice Guideline for Allergic Rhinitis.* 4th edn. Tokyo:Lifescience, 2002.
14. Okuda M, Okubo K, Gotoh M *et al.* Japanese Allergic Rhinitis QOL standard questionnaire (2002). *Arerugi* 2003;**52**:21-56(in Japanese).
15. Kimura I, Tanizaki Y, Goda Y, Komagoe H, Kitani H. Decrease in reactivity of basophils by immunotherapy with housedust extract. *Clin. Allergy* 1985;**15**:1-7.
16. Otsuka H, Mezawa A, Ohnishi M, Okubo K, Seki H, Okuda M. Changes in nasal metachromatic cells during allergen immunotherapy. *Clin. Exp. Allergy* 1991;**21**:115-119.
17. Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J. Allergy Clin. Immunol.* 1998;**102**:157-164.
18. van Neerven RJ, Wikborg T, Lund G *et al.* Blocking antibodies induced by specific allergy vaccination prevent the activation of CD 41 T cells by inhibiting serum-IgE-facilitated allergen presentation. *J. Immunol.* 1999;**163**: 2944-2952.
19. Golden DB, Meyers DA, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Clinical relevance of the venom-specific immunoglobulin G antibody level during immunotherapy. *J. Allergy Clin. Immunol.* 1982;**69**:489-493.
20. Van-der-Zee JS, Aalberse RC. The role of IgG in immediate-type hypersensitivity. *Eur. Respir. J. Suppl.* 1991;**13**:91-96s.
21. Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J. Exp. Med.* 1993;**178**:2123-2130.
22. Lamb JR, Skidmore BJ, Green N, Chiller JM, Feldmann M. Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. *J. Exp. Med.* 1983;**157**:1434-1447.
23. Fasler S, Aversa G, Terr A, Thestrup-Pedersen K, De Vries JE, Yssel H. Peptide-induced anergy in allergen-specific human Th2 cells results in lack of cytokine production and B cell for IgE synthesis : reversal by IL-2, not by IL-4 or IL-13. *J. Immunol.* 1995;**155**:4199-4206.
24. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-γ secretion in specific allergen-stimulated T cell cultures. *J. Immunol.* 1995;**154**:4187-4194.
25. Muller D, Ruitenberg EJ, Elgersma A. The influence of different immunization pathways on the immunological response in the oral mucosa. *Br. J. Exp. Pathol.* 1983;**64**: 367-372.
26. Bagnasco M, Passalacqua G, Villa G *et al.* Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin. Exp. Allergy* 2001;**31**:54-60.
27. World Health Organization. Allergen immunotherapy : therapeutic vaccines for allergic diseases(WHO position paper). *J. Allergy Clin. Immunol.* 1998;**102**:558-562.
28. Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. *Ann. Allergy Asthma Immunol.* 2003; **91**:288-296.

アレルギー性鼻炎の疫学
——2005年の調査から
Epidemiology of allergic rhinitis in Japan



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◎アレルギー性鼻炎患者の増加が指摘されているが，実態はかならずしも明らかではなく，国内での疫学調査は不足している．2005年のスギ・ヒノキ花粉飛散終了後に行った山梨と千葉でのアレルギー性鼻炎の検診をもとに検討を行った．わが国特有ともいえるスギ花粉症であるが，スギ花粉に対する感作率・発症率の上昇は小児から中・高年者まで広い年齢層でみられ，2005年のようなスギ・ヒノキ花粉の大量飛散はこの傾向を助長している．小学生でもスギ花粉に対する感作率は60%，発症率は40%を超えており，増加が著しい．ただ，関東地方で異なる花粉飛散数を示す地域での調査から，花粉飛散のみでなく，児童の“体質”に変化を及ぼす他の因子の関与が想定される．一方，ダニに対する感作についても小児で増加しているが，中・高年では変化していない．ただ，小児，成人とも重複感作の割合は高く，小児スギ花粉感作陽性者の約80%がダニに重複感作を示している．

Key word：アレルギー性鼻炎，疫学調査，花粉症，小児，成人

アレルギー性鼻炎の増加は世界的にみられるが[1-3]，国内では大規模な疫学調査が不足している．とくに，わが国特有といえるスギ花粉症についてまとまった報告は少ない．有効な対策の確立にはアレルギー性鼻炎の現状の正確な把握が重要なことはいうまでもないが，アレルギー性鼻炎に関する疫学調査は不足しており，加えて最近は調査そのものの実施が以前に比べ難しくなっている．限られた検討対象数ではあるが，全体を把握するのに十分な意義があると考え，2005年に著者らが行いえたアレルギー性鼻炎に関する調査を中心にまとめてみたい．

アレルギー性鼻炎の疫学調査の注意点・問題点

調査対象の住民，患者あるいは児童，その保護者に調査の目的と意義を十分に説明し文書での同意を得ることはいうまでもないが，実際の調査では市町村役所，地区医師会，児童の場合には教育委員会，学校長，養護教員，学校医の理解・協力が不可欠である．さらに，得られたデータ・知見を広くフィードバックする必要があり，この連携を十分に保っておかないと調査の継続は困難となる．

アンケートによる調査で，たとえば「かぜでもないのにくしゃみ，鼻汁，鼻づまりが続くことがありますか」との質問では，多くのfalse positiveがみられることが知られている．世界的規模で行われたISSACのアレルギー性鼻炎の有症率の調査では，途上国でも異様に高い有症率が報告されている[4]．精度を高めるためには皮膚テスト，あるいは血清中特異IgE抗体の測定が不可欠である．もちろん，アレルゲン特異的IgE抗体が低値でも発症している患者もいることは事実であり，このような患者はIgE抗体陽性を必要条件とする場合には患者として漏れてしまう可能性があるが，アンケート調査からだけの判断ではあまりに不正確である．さらに可能であれば，シーズン中の鼻内

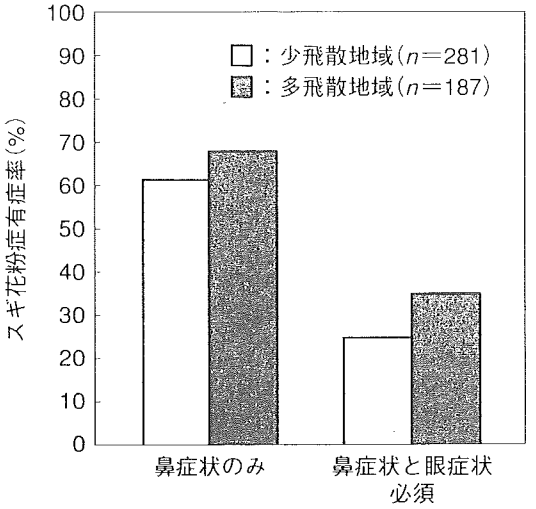

図1　アンケート結果のみから推測したスギ花粉症有症率（鼻症状のみの場合と鼻症状と眼症状をともに必須とした場合の比較）

児童の保護者へのアンケート調査で2月から4月のくしゃみ，鼻みず，鼻づまり，あるいは眼のかゆみ，充血などが児童にみられるかという問いに対する回答を示す．鼻の症状のみを考慮すると有症率は非常に高い割合になってしまう．

診察や鼻汁細胞診，問診を加えることで診断の精度は向上するが，実際の検診・調査では困難であり，通常はアンケート，あるいは問診と血清中特異IgE抗体から判断することになる．

一方，乳幼児では鼻症状に対する親の関心のあり方も大きく調査結果に影響する．喘息やアトピー性皮膚炎の症状には敏感でも，鼻みずや鼻閉に対する関心は低いことが多く，医師にとっても症状の把握が困難なことが多い．また，小児は一般に鼻内所見と訴えとの間に乖離がみられる．診断の精度を高めるためには，鼻内所見や鼻汁細胞診，詳細な問診が有用であり，とくに年少児では価値が高い．もちろん，多数の患児を対象にした検診では難しいが，患児を経時的にフォローする場合には，やはりこのような精度の高い診断が必要である．

逆に過敏に反応してしまう場合もある．とくに検診などで保護者にアレルギー性鼻炎の増加を指摘して調査の同意を求めるためか，非常に高い児童の“有症率”がみられることがある（図1）．

小児の調査から

2005年のスギ・ヒノキ花粉飛散終了後の6月に，山梨県の農村部の小学校においてアレルギー性鼻炎の検診を行った．小学生のアレルギー性鼻炎の実態を調べるのみでなく，例年花粉飛散の多い地域（山梨県南部富河地区）と飛散が比較的少ない地域（山梨県北杜地区）のそれぞれ2つの小学校で花粉飛散数が異なる環境で成育した児童のアレルギー性鼻炎の発症の違いを明らかにすることを目的としたが，ちなみに2つの地域での花粉飛散はダーラム法で1998～2003年の平均で約6,700個/cm^2/シーズンと2,200個/cm^2/シーズンと，3倍の違いがみられる．4校の総生徒数は485名で，このうちアンケート同意率は97%，採血同意率は68%で，採血不同意者のなかにはすでに検査を受

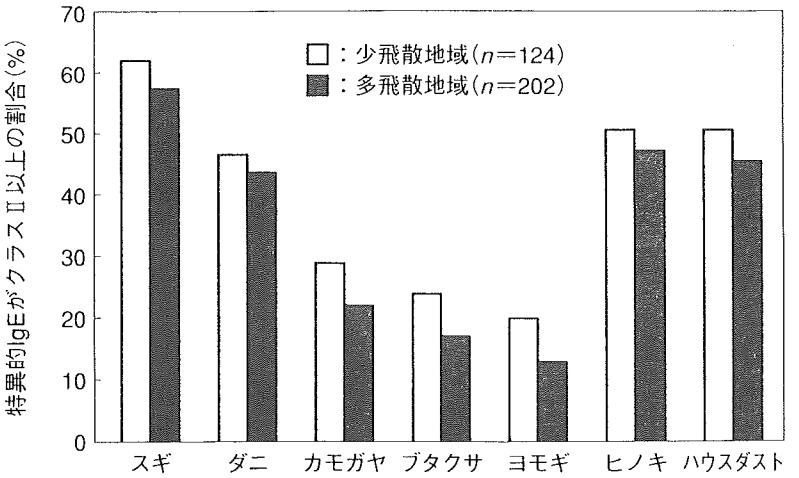

図2　2005年小学生の調査（投稿準備中）

花粉飛散数の異なる2地域での各種抗原感作率の比較．小学生の代表的アレルゲンに対する感作率（CAP RAST2以上）を示す．

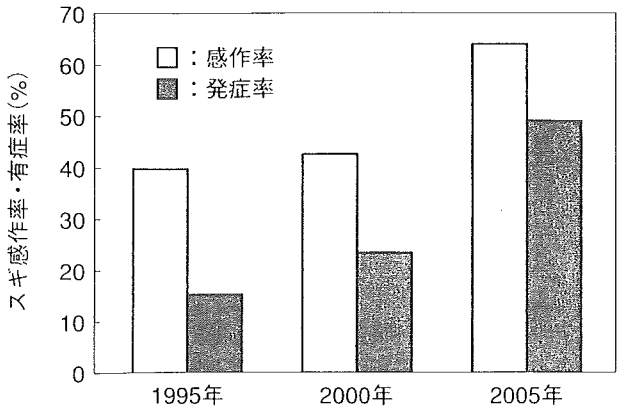

図 3　中学生スギ感作率・有症率(千葉県安房郡丸山町)(投稿準備中)
同一中学校での各種アレルゲンに対する感作率の2005年と2000年，1995年(いずれも6月)の比較．

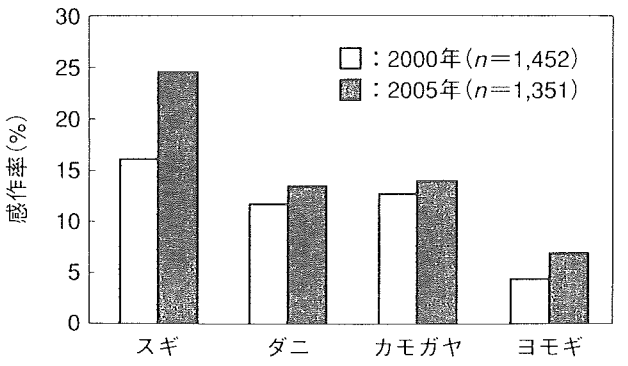

図 4　感作率の変化(成人)(投稿準備中)
同一町村の40歳以上の成人の各種アレルゲンに対する感作率の2005年と2000年(いずれも6月)の比較

け通院中の児童もおり，アンケート結果から採血同意者・不同意者で有症率に有意差は認めなかった．また，2つの地域で男女数に差は認めなかった．

図2は代表的アレルゲンに対する感作率を示すが，スギは約60%，ダニ50%と高く，そのほかヒノキ約50%，カモガヤ25%，ブタクサ20%，ヨモギ18%といった感作率がみられた．スギ花粉感作者のうちスギ花粉飛散時期に一致した鼻症状ならびに眼症状を訴える児童の割合，すなわち発症率は40%を超えていた．また，スギ花粉感作児童のうち重複感作はダニに60%，カモガヤに40%みられた．このように小学生のアレルゲンに対する感作率・発症率は高く，たとえばスギに対しては感作率60%，発症率40%と2001年の千葉県市川市，君津市の小学生での調査結果[5](感作率37%，発症率24%)と比較して高い数字となっている．また，全IgE値も重複感作例では単独感作例に比較して高値を示している．ただ，花粉飛散数の違いによる地域差は明らかではなかった．このことから，とくにスギ花粉の感作率の上昇が著しいが，花粉飛散の増加だけではなく，小学生の“体質”の変化も大きく影響していることが推察される．

一方，図3は千葉県安房郡丸山町での同一中学校(1，2年生)を対象にしたアンケート，ならびにスギ・ヒノキ花粉飛散終了後5～6月の血清中の特異IgE抗体の2005年と2000年，1995年の比較を示す．スギをはじめ，いずれの代表的アレルゲンに対する感作率も2000年と比較して2005年では増加を示している．

小学生未満の年少児での正確なアレルゲン感作率・発症率は不明である．河野らは，小児喘息患者では80%と高率にアレルギー性鼻炎の合併が疑われること，合併している児童の保護者のうち約20%が喘息よりさきにアレルギー性鼻炎が発症していたという印象をもっており，その場合のアレルギー性鼻炎の平均発症年齢は1.4歳と低いことを報告している[6]．著者らが千葉大学小児科アレルギー外来通院中の小児を調査したところ，喘息児31名(平均年齢5.7歳)では71%にアレルギー性鼻炎の合併を，また喘息のないアトピー性皮膚炎あるいは食物アレルギーの患児23名中39%にアレルギー性鼻炎を認めた．喘息児のみならず，非喘息のアトピー疾患患児にも高い割合で，かつ低年齢からアレルギー性鼻炎の合併が認められる．

成人での調査から

当科では1995年以来毎年房総半島南端に位置する安房郡丸山町(全人口約5,700名)で住民のアレルギー性鼻炎の検診を行っている[7]．検診はスギ・ヒノキ花粉飛散終了後の6月にアンケート調査，問診，血清中特異的IgE抗体検査により行われ，毎年40歳代以降を中心に1,300～1,600名が参加する．図4は2000年と2005年の代表的アレルゲンに対する感作率を比較したものであるが，スギは感作率の上昇がみられるが，他はほぼ横ばい