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EDITORIAL

Strategy for prevention of hip fractures in patients with Parkinson's disease

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Abstract

Hypovitaminosis D and K due to malnutrition or sunlight deprivation, increased bone resorption due to immobilization, low bone mineral density (BMD) and an increased risk of falls may contribute to an increased risk of hip fractures in patients with Parkinson's disease. The purpose of the present study was to clarify the efficacy of interventions intended to prevent hip fractures in elderly patients with Parkinson's disease. PubMed was used to search the literature for randomized controlled trials (RCTs) regarding Parkinson's disease and hip fractures. The inclusion criteria were 50 or more subjects per group and a study period of 1 year or longer. Five RCTs were identified and the relative risk and 95% confidence interval were calculated for individual RCTs. Sunlight exposure increased serum hydroxyvitamin D [25(OH)D] concentration, improved motor function, decreased bone resorption and increased BMD. Alendronate or risedronate with vitamin D supplementation increased serum 25(OH)D concentration, strongly decreased bone resorption and increased BMD. Menatetrenone (vitamin K_2) decreased serum undercarboxylated osteocalcin concentration, decreased bone resorption and increased BMD. Sunlight exposure (men and women), menatetrenone (women), alendronate and risedronate with vitamin D supplementation (women) significantly reduced the incidence of hip fractures. The respective RRs (95% confidence intervals) according to the intention-to-treat analysis were 0.27 (0.08, 0.96), 0.13 (0.02, 0.97), 0.29 (0.10, 0.85) and 0.20 (0.06, 0.68). Interventions, including sunlight exposure, menatetrenone and oral bisphosphonates with vitamin D supplementation, have a protective effect against hip fractures elderly patients with Parkinson's disease.

Key words: Vitamin D; Vitamin K; Hip fractures; Parkinson's disease; Mortality

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INTRODUCTION

Parkinson's disease is a movement disorder characterized by tremor, rigidity, akinesia and loss of postural reflexes, leading to immobility and frequent falls[1,2]. Evidence has indicated a high incidence of hip fractures in patients with Parkinson's disease, with falls being a major cause[3-5]. This is especially true in elderly women and the odds ratio of hip fractures in elderly women is reported to be 9.4[5,6]. Hip fractures are associated with higher medical

costs[7]. Functional recovery after hip fractures in patients with osteoporosis is poor[8-10] and elderly patients have increased mortality after hip fractures[11]. Thus, strategies protecting against hip fractures should be established in elderly patients with Parkinson's disease.

Hypovitaminosis D and K due to malnutrition or sunlight deprivation, increased bone resorption due to immobilization, low bone mineral density (BMD) and an increased risk of falls may increase the risk of hip fractures in elderly patients with Parkinson's disease[12-16]. Hypovitaminosis D is known to increase the risk of falls in the elderly[17-19]. An immobilization-induced increase in bone resorption causes hypercalcemia, which may inhibit the compensatory hyperparathyroidism that otherwise could occur in response to hypovitaminosis D. Sunlight exposure, vitamin D and K supplementation, and potent anti-resorptive drugs are considered to be effective strategies to prevent hip fractures. Recent evidence has shown the efficacy of interventions protective against hip fractures in elderly patients with Parkinson's disease[12-16]. The purpose of the present study was to clarify the efficacy of these interventions in elderly patients with Parkinson's disease by reviewing the literature to date.

LITERATURE SEARCH

PubMed was used to search the literature for randomized controlled trials (RCTs) of interventions affecting the incidence of hip fractures in patients with Parkinson's disease. The following terms were used: Parkinson's disease and fracture. The inclusion criteria were 50 or more subjects per group and a study period of 1 year or longer. Non-English papers were excluded.

RCTs showing efficacy of interventions against hip fractures were identified and the efficacy of interventions against hip fractures was analyzed using the data from the RCTs. The relative risk (RR) and 95% confidence interval (CI) were calculated for individual trials. The statistical analyses were performed using PC SAS v8.2.

IDENTIFIED RANDOMIZED CONTROLLED TRIALS

Five RCTs were found dealing with Parkinson's disease and hip fractures[12-16]. Table 1 shows the details of the identified RCTs: one RCT for sunlight exposure, one RCT for menatetrenone (vitamin K_2), one RCT for alendronate and two RCTs for risedronate. All of the RCTs were performed in Japan. The mean ages of the subjects were 71.3-75.4 years, reflecting studies in the elderly population. The mean durations of their illness (Parkinson's disease) were 4.1-5.1 years. The studies lasted for 1-2 years. Patients were exposed to sunlight on 452 clear weather days (3231 min/year) during the 2 year study period. The doses of menatetrenone (45 mg/d), alendronate (5 mg/d) and risedronate (2.5 mg/d or 17.5 mg/wk) used in the RCTs were approved by the Health, Labor and Welfare Ministry of Japan. Calcium supplementation was not provided in any RCT because such a therapy could aggravate immobilization-induced hypercalcemia and decrease renal synthesis of 1,25 dihydroxyvitamin D [1,25$(OH)_2$D]. Vitamin D (ergocalciferol 1000 IU/d) supplementation was provided in three RCTs for alendronate and risedronate (potent anti-resorptive drugs). During the trials, 4.3%-9.7% of patients were dropped because of death or intercurrent illness, noncompliance or loss to follow-up. No severe adverse events were observed.

EFFICACY OF SUNLIGHT EXPOSURE AGAINST HIP FRACTURES

Study subjects were men and women. Serum 25(OH)D concentration, muscle strength, motor function and metacarpal BMD increased in the sunlight exposure group and decreased in the usual lifestyle group[12]. Urinary deoxypyridinoline concentration decreased in the sunlight exposure group and increased in the usual lifestyle group. Respective changes in serum 25(OH)D concentration were +92.6% and -51.9%. Respective percentage changes in metacarpal BMD were +3.8% and -2.6%. The RR (95% CI) for hip fractures in the sunlight exposure group compared with the usual lifestyle group was 0.27 (0.08, 0.95) for the intent-to-treat (ITT) set and 0.27 (0.08, 0.95) for the per protocol set (PPS) (Table 2), suggesting a significant reduction in the risk of hip fractures after sunlight exposure therapy.

EFFICACY OF MENATETRENONE AGAINST HIP FRACTURES

Study subjects were women. Serum vitamin K_2 concentration increased and serum undercarboxylated osteocalcin (ucOC) decreased in the menatetrenone group compared with the non-treatment group[13]. Respective changes in serum vitamin K_2 concentration were +259.8% and -1.8%. Respective changes in serum ucOC concentration were -46.7% and +3.3%. Urinary deoxypyridinoline and serum ionized calcium concentrations decreased, intact PTH concentrations increased and metacarpal BMD increased in the menatetrenone group compared with the non-treatment group. Respective percentage changes in metacarpal BMD were +0.9% and -4.3%. The RR (95% CI) of hip fractures after menatetrenone treatment compared with non-treatment was 0.13 (0.02, 0.97) for the ITT set and 0.12 (0.02, 0.93) for the PPS (Table 2), suggesting a significant reduction in the risk for hip fractures after menatetrenone therapy.

EFFICACY OF ALENDRONATE AGAINST HIP FRACTURES

Study subjects were women. Serum 25(OH)D concentra-

Table 1 Identified randomized controlled trials of efficacy of interventions against hip fractures in patients with Parkinson's disease

Interventions	Groups	Number of study subjects			Average age (yr)	Average duration of illness (yr)	Vitamin D supplementation	Study period (yr)
		Randomized	Dropped out	Completed				
Sunlight exposure[12]	Sunlight exposure	162	6	156	75.4	4.2	None	2
(men/women)	Usual lifestyle	162	8	154	75.2	4.1		
menatetrenone[13]	Menatetrenone	60	4	56	72.3	4.8	None	1
(women)	Non-treatment	60	6	54	71.6	4.9		
Daily alendronate[14]	Alendronate	144	13	131	72.2	5.1	Ergocalciferol (1000 IU/d)	2
(women)	Placebo	144	15	129	72.2	5.1		
Daily risedronate[15]	Risedronate	121	10	111	71.3	4.9	Ergocalciferol (1000 IU/d)	2
(men)	Placebo	121	9	112	71.3	4.9		
Weekly risedronate[16]	Risedronate	136	10	126	74.4	4.8	Ergocalciferol (1000 IU/d)	2
(women)	Placebo	136	12	124	74.4	4.9		

Table 2 Efficacy of interventions against hip fractures in patients with Parkinson's disease

Interventions	Relative risk (95% confidence interval)	
	ITT set	PPS
Sunlight exposure[12]	0.27 (0.08, 0.96)	0.27 (0.08, 0.95)
Menatetrenone[13]	0.13 (0.02, 0.97)	0.12 (0.02, 0.93)
Alendronate (Daily)[14]	0.29 (0.10, 0.85)	0.28 (0.10, 0.83)
Risedronate (Daily)[15]	0.33 (0.09, 1.20)	0.34 (0.09, 1.21)
Risedronate (Weekly)[16]	0.20 (0.06, 0.68)	0.20 (0.06, 0.66)

ITT: Intention-to-treat, PPS: Per-protocol set.

tion increased, urinary deoxypyridinoline and serum ionized calcium concentrations decreased, and metacarpal BMD increased in the alendronate + vitamin D supplementation group[14]. Serum 25(OH)D, urinary deoxypyridinoline and serum ionized calcium concentrations increased, and metacarpal BMD decreased in the placebo + vitamin D supplementation group. Respective changes in serum 25(OH)D concentration were +209.8% and +209.5%. Respective changes in urinary deoxypyridinoline concentration were -38.1% and +14.0%. Respective percentage changes in metacarpal BMD were +3.1% and -2.8%. The RR (95% CI) of hip fractures after alendronate compared with placebo was 0.29 (0.10, 0.85) for the ITT set and 0.28 (0.10, 0.83) for the PPS (Table 2), suggesting a significant reduction in the risk of hip fractures after alendronate therapy with vitamin D supplementation.

EFFICACY OF RISEDRONATE AGAINST HIP FRACTURES

Study subjects were men for the daily risedronate study and women for the weekly risedronate study[15,16]. Changes in serum 25(OH)D, urinary deoxypyridinoline, serum ionized calcium concentrations and metacarpal BMD in the two studies of daily and weekly risedronate + vitamin D supplementation (compared with placebo + vitamin D supplementation) were similar to those in the study of alendronate + vitamin D supplementation (compared with placebo + vitamin D supplementation) shown above. Respective changes in serum 25(OH)D concentration were +198.4% to +211.1% and +185.2% to +198.4%. Respective changes in urinary deoxypyridinoline concentration were -48.2% to -50.4% and +18.3% to +19.2%. Respective percentage changes in metacarpal BMD were +2.2% to +3.4% and -2.9% to -3.2%. The RR (95% CI) of hip fractures after daily risedronate compared with placebo in men was 0.33 (0.09, 1.20) for the ITT set and 0.34 (0.09, 1.21) for the PPS (Table 2). The RR (95% CI) of hip fractures after daily risedronate compared with placebo in women was 0.20 (0.06, 0.68) for the ITT set and 0.20 (0.06, 0.66) for the PPS (Table 2). These results suggested a significant reduction in the risk for hip fractures after risedronate therapy with vitamin D supplementation in elderly women with Parkinson's disease.

DISCUSSION

The present study clarified the efficacy of interventions (including sunlight exposure, menatetrenone and oral bisphosphonates with vitamin D supplementation) protecting against hip fractures in elderly patients with Parkinson's disease. Because hypovitaminosis D and K, increased bone resorption, low BMD and an increased risk of falls contribute to the risk for hip fractures in elderly patients with Parkinson's disease[12-16], these three interventions were suggested to be reasonable and effective for the management of bone health.

BMD, thickness, porosity and mean degree of mineralization in cortical bone may be important factors in determining the fracture risk at sites primarily composed of cortical bone such as the proximal femur in postmenopausal women with osteoporosis[20,21]. Because most hip fractures occur due to falls, motor function may also be an important factor in the risk of hip fractures. Serum 25(OH)D is derived from both dietary intake and sunlight-induced production by the skin[22,23]. The associations of hypovitaminosis D and vitamin D supplementation with the risk of falls have been confirmed in elderly persons[17-19]. Sunlight exposure improves hypovi-

taminosis D, leading to increases in muscle strength and motor function in men and women. A decrease in bone resorption induces an increase in cortical BMD. It is documented that cortical BMD correlates positively with serum 25(OH)D concentration, particularly in the subjects with vitamin D insufficiency[24]. Thus, improvements of muscle strength, motor function and cortical BMD might partly contribute to the prevention of hip fractures. Sunlight exposure appears to help prevent hip fractures in patients with Parkinson's disease and hypovitaminosis D due to malnutrition and sunlight deprivation.

Vitamin K deficiency, as indicated by a high serum ucOC concentration, and low BMD may independently contribute to the risk for hip fractures in elderly persons[25-27]. Menatetrenone improved hypovitaminosis K, decreased serum ucOC concentration, improved hypercalcemia and increased cortical BMD by decreasing bone resorption in women. Experimental studies showed the anti-resorptive effect of menatetrenone in various osteoporosis model animals[28,29]. A recent report showed that menatetrenone maintains bone strength of the femoral neck by improving femoral neck width and maintaining the indices of compression, bending and impact strength in healthy postmenopausal women[30]. Thus, improvements of cortical BMD, serum ucOC concentration and possibly bone geometry of the proximal femur might have partly contributed to the prevention of hip fractures. Menatetrenone appeared to be effective in preventing hip fractures in patients with Parkinson's disease and hypovitaminosis K. However, the magnitude of hip fracture risk reduction was quite high, probably because of the bias introduced by the use of a small sample size and the low intake of natto (fermented soy bean), in terms of severe vitamin K deficiency in the recruited subjects[31].

Alendronate or risedronate with vitamin D supplementation improved hypovitaminosis D, strongly decreased bone resorption, improved hypercalcemia and increased cortical BMD in men or women. Alendronate has been reported to strongly suppress bone resorption and improve femoral neck BMD, cortical thickness, cortical porosity and mean degree of mineralization of bone and thereby to prevent hip fractures in postmenopausal women with osteoporosis[20,21]. The greater the suppression of bone turnover and subsequent increase in BMD are, the better the drugs are at preventing nonvertebral fractures, including hip fractures[32]. Thus, improvements in the above parameters resulting from strong suppression of bone resorption[21] and a decrease in the risk of falls by vitamin D supplementation[17-19] may partly contribute to the prevention of hip fractures in women. Alendronate or risedronate and vitamin D supplementation appear to be quite effective for preventing hip fractures in women with Parkinson's disease and hypovitaminosis D, as well as increased bone resorption. However, risedronate and vitamin D supplementation did not significantly reduce the incidence of hip fractures in men, probably because of less than adequate statistical power due to the lower incidence of hip fractures in men (7.4% in the placebo + vitamin D supplementation group) compared with women (11.0% in the placebo + vitamin D supplementation group).

During the trials, 4.3-9.7% of patients were dropped because of death or intercurrent illness, noncompliance or loss to follow-up. However, no severe adverse events were observed, suggesting the safety of all interventions (sunlight exposure and pharmacotherapy such as menatetrenone and oral bisphosphonates) in elderly patients with Parkinson's disease.

Because patients with Parkinson's disease are prone to falls, not only sunlight exposure or vitamin D supplementation, but also hip protectors and exercise aiming at the prevention of falls may help reduce the incidence of hip fractures. However, exercise therapy may be difficult for patients with very advanced Parkinson's disease. Further studies are needed to confirm this suggestion.

CONCLUSION

The present study clarified the efficacy of three interventions, including sunlight exposure (men and women), menatetrenone (women) and oral bisphosphonates with vitamin D supplementation (women), protective against hip fractures in patients with Parkinson's disease. The risk of hip fractures was reduced 73% by sunlight exposure, 87% by menatetrenone treatment and 71-80% by oral bisphosphonate treatment. The efficacy of exercise and hip protectors remains to be established. These interventions might be difficult to perform but may help reduce the incidence of falls and possibility of hip fractures.

REFERENCES

1 **Aita JF**. Why patients with Parkinson's disease fall. *JAMA* 1982; **247**: 515-516
2 **Koller WC**, Glatt S, Vetere-Overfield B, Hassanein R. Falls and Parkinson's disease. *Clin Neuropharmacol* 1989; **12**: 98-105
3 **Chiu KY**, Pun WK, Luk KD, Chow SP. Sequential fractures of both hips in elderly patients--a prospective study. *J Trauma* 1992; **32**: 584-587
4 **Johnell O**, Melton LJ, Atkinson EJ, O'Fallon WM, Kurland LT. Fracture risk in patients with parkinsonism: a population-based study in Olmsted County, Minnesota. *Age Ageing* 1992; **21**: 32-38
5 **Grisso JA**, Kelsey JL, Strom BL, Chiu GY, Maislin G, O'Brien LA, Hoffman S, Kaplan F. Risk factors for falls as a cause of hip fracture in women. The Northeast Hip Fracture Study Group. *N Engl J Med* 1991; **324**: 1326-1331
6 **Eventov I**, Moreno M, Geller E, Tardiman R, Salama R. Hip fractures in patients with Parkinson's syndrome. *J Trauma* 1983; **23**: 98-101
7 **Nurmi I**, Narinen A, Lüthje P, Tanninen S. Cost analysis of hip fracture treatment among the elderly for the public health services: a 1-year prospective study in 106 consecutive patients. *Arch Orthop Trauma Surg* 2003; **123**: 551-554
8 **Holmes J**, House A. Psychiatric illness predicts poor outcome after surgery for hip fracture: a prospective cohort study. *Psychol Med* 2000; **30**: 921-929
9 **Matsueda M**, Ishii Y. The relationship between dementia score and ambulatory level after hip fracture in the elderly. *Am J Orthop* (*Belle Mead NJ*) 2000; **29**: 691-693
10 **Morrison RS**, Siu AL. Mortality from pneumonia and hip

fractures in patients with advanced dementia *JAMA* 2000; **284**: 2447-2448
11 **Nightingale S**, Holmes J, Mason J, House A. Psychiatric illness and mortality after hip fracture. *Lancet* 2001; **357**: 1264-1265
12 **Sato Y**, Iwamoto J, Honda Y. Amelioration of osteoporosis and hypovitaminosis D by sunlight exposure in Parkinson's disease. *Parkinsonism Relat Disord* 2011; **17**: 22-26
13 **Sato Y**, Honda Y, Kaji M, Asoh T, Hosokawa K, Kondo I, Satoh K. Amelioration of osteoporosis by menatetrenone in elderly female Parkinson's disease patients with vitamin D deficiency. *Bone* 2002; **31**: 114-118
14 **Sato Y**, Iwamoto J, Kanoko T, Satoh K. Alendronate and vitamin D2 for prevention of hip fracture in Parkinson's disease: a randomized controlled trial. *Mov Disord* 2006; **21**: 924-929
15 **Sato Y**, Honda Y, Iwamoto J. Risedronate and ergocalciferol prevent hip fracture in elderly men with Parkinson disease. *Neurology* 2007; **68**: 911-915
16 **Sato Y**, Iwamoto J, Honda Y. Once-weekly risedronate for prevention of hip fracture in women with Parkinson's disease: a randomised controlled trial. *J Neurol Neurosurg Psychiatry* 2011; **82**: 1390-1393
17 **Sato Y**, Inose M, Higuchi I, Higuchi F, Kondo I. Changes in the supporting muscles of the fractured hip in elderly women. *Bone* 2002; **30**: 325-330
18 **Bischoff-Ferrari HA**, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, Wong JB. Effect of Vitamin D on falls: a meta-analysis. *JAMA* 2004; **291**: 1999-2006
19 **Bischoff-Ferrari HA**, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA* 2005; **293**: 2257-2264
20 **Epstein S**. Is cortical bone hip? What determines cortical bone properties? *Bone* 2007; **41**: S3-S8
21 **Iwamoto J**, Sato Y, Takeda T, Matsumoto H. Hip fracture protection by alendronate treatment in postmenopausal women with osteoporosis: a review of the literature. *Clin Interv Aging* 2008; **3**: 483-489
22 **Beadle PC**. Sunlight, ozone and vitamin D. *Br J Dermatol* 1977; **97**: 585-591
23 **Lester E**, Skinner RK, Foo AY, Lund B, Sørensen OH. Serum 25-hydroxyvitamin D levels and vitamin D intake in healthy young adults in Britain and Denmark. *Scand J Clin Lab Invest* 1980; **40**: 145-150
24 **Sato Y**, Metoki N, Iwamoto J, Satoh K. Amelioration of osteoporosis and hypovitaminosis D by sunlight exposure in stroke patients. *Neurology* 2003; **61**: 338-342
25 **Szulc P**, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. *J Clin Invest* 1993; **91**: 1769-1774
26 **Szulc P**, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study. *Bone* 1996; **18**: 487-488
27 **Vergnaud P**, Garnero P, Meunier PJ, Bréart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. *J Clin Endocrinol Metab* 1997; **82**: 719-724
28 **Iwamoto J**, Takeda T, Sato Y. Effects of vitamin K2 on osteoporosis. *Curr Pharm Des* 2004; **10**: 2557-2576
29 **Iwamoto J**, Takeda T, Sato Y. Effects of vitamin K2 on the development of osteopenia in rats as the models of osteoporosis. *Yonsei Med J* 2006; **47**: 157-166
30 **Knapen MH**, Schurgers LJ, Vermeer C. Vitamin K2 supplementation improves hip bone geometry and bone strength indices in postmenopausal women. *Osteoporos Int* 2007; **18**: 963-972
31 **Yaegashi Y**, Onoda T, Tanno K, Kuribayashi T, Sakata K, Orimo H. Association of hip fracture incidence and intake of calcium, magnesium, vitamin D, and vitamin K. *Eur J Epidemiol* 2008; **23**: 219-225
32 **Hochberg MC**, Greenspan S, Wasnich RD, Miller P, Thompson DE, Ross PD. Changes in bone density and turnover explain the reductions in incidence of nonvertebral fractures that occur during treatment with antiresorptive agents. *J Clin Endocrinol Metab* 2002; **87**: 1586-1592

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TOPIC HIGHLIGHT

Hisataka Yasuda, PhD, *Series Editor*

RANKL-RANK interaction in immune regulatory systems

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Abstract

The interaction between the receptor activator of NF-κB ligand (RANKL) and its receptor RANK plays a critical role in the development and function of diverse tissues. This review summarizes the studies regarding the functions of RANKL signaling in immune regulatory systems. Previous *in vitro* and *in vivo* studies have indicated that the RANKL signal promotes the survival of dendritic cells (DCs), thereby activating the immune response. In addition, RANKL signaling to DCs in the body surface barriers controls self-tolerance and oral-tolerance through regulatory T cell functions. In addition to regulating DC functions, the RANKL and RANK interaction is critical for the development and organization of several lymphoid organs. The RANKL signal initiates the formation of clusters of lymphoid tissue inducer cells, which is crucial for lymph node organogenesis. Moreover, the RANKL-RANK interaction controls the differentiation of M cells, specialized epithelial cells in mucosal tissues, that take up and transcytose antigen particles to control the immune response to pathogens or commensal bacterium. The development of epithelial cells localized in the thymic medulla (mTECs) is also regulated by the RANKL-RANK signal. Given that the unique property of mTECs to express a wide variety of tissue-specific self-antigens is critical for the elimination of self-antigen reactive T cells in the thymus, the RANKL-RANK interaction contributes to the suppression of autoimmunity. Future studies on the roles of the RANKL-RANK system in immune regulatory functions would be informative for the development and application of inhibitors of RANKL signaling for disease treatment.

Key words: RANKL; T cells; Dendritic cells; Thymus; Medullary thymic epithelial cells; Lymphoid tissue inducer cells; Lymph node; M cells; Peyer's patches

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INTRODUCTION

The tumor necrosis factor (TNF) family member receptor activator of NF-κB ligand (RANKL) and its receptor RANK regulate diverse physiological functions and organ development in the body. Because RANKL- and RANK-deficient mice exhibit severe osteopetrosis, multiple studies have focused on the role of the RANKL-RANK axis and its underlying mechanism in the regulation of bone homeostasis through osteoclast differentiation. As a result of these extensive studies, a fully humanized anti-RANKL neutralizing antibody has been developed and recently approved for the treatment of osteoporosis,

rheumatoid arthritis and cancer bone metastasis. However, RANKL and RANK were originally identified as a cytokine and its receptor, respectively, that control the function of dendritic cells (DCs)[1,2] in addition to their role in osteoclast differentiation[3]. Moreover, RANKL- and RANK-deficient mice (RANKL-KO and RANK-KO) manifest defects in the development, organization and function of several lymphoid organs[2,4-6]. In this review, we summarize the current knowledge regarding the roles of RANKL/RANK signaling in immune regulatory systems, including immune activation, immunological tolerance and lymphoid organogenesis.

DENDRITIC CELLS FUNCTIONS

Activation of T cells

RANK shows a relatively high homology with CD40 among the TNF receptor family members[2]. The cytoplasmic region of RANK contains binding sites for the tumor necrosis factor receptor-associated factor (TRAF) family proteins TRAF2, 3, 5 and 6[7-10], which is a structural property shared with CD40[11]. These TRAF family proteins mediate the activation of the NF-κB and MAPK pathways to result in the transcriptional activation of genes required for proliferation, survival and differentiation[7,11]. Therefore, it is reasonable to assume that RANK signaling initiates cellular responses similar to CD40 signaling by triggering these common intracellular signaling pathways. A large number of studies have revealed that CD40 signaling induces the maturation, activation and survival of DCs[12,13]. DCs are classified into several subsets according to their functions, localizations and cell surface markers[14]. Conventional DCs (cDCs) are the prototypical professional antigen-presenting cells that engulf, process and present antigens, thereby priming and activating T cells for the initiation of the acquired immune response. The RANK cDNA was discovered by the direct sequencing of a cDNA library prepared from cDCs. In addition, RANKL expression is upregulated in activated T cells[15]. Therefore, early studies analyzed the effects of RANKL treatment on the ability of cDCs to prime T cells *in vitro*. Indeed, two independent studies revealed that RANKL treatment promotes the survival of cDCs, thereby efficiently priming T cells[2,16] (Figure 1). Interestingly, whereas CD40 signaling upregulates the surface expression of the co-stimulatory molecules CD80/86 and the major histocompatibility complex (MHC) molecules, RANK signaling does not[2,16]. Thus, although RANK and CD40 activate similar intracellular signaling pathways, the outputs of these two signals in the cells are slightly different. The "adjuvant" effect of the RANKL signal was further confirmed by *ex vivo* transfer of cDCs treated with RANKL[17]. The impact of RANK deficiency on DC function has not been clearly defined in RANKL-KO and RANK-KO mice, most likely because these null mutant mice manifest complicated and combined phenotypes and die at young ages after birth. However, it has been shown that blocking the RANKL-RANK interaction by injecting non-membrane-bound RANK-Fc protein suppresses $CD4^+$ T cell-mediated immune activation induced by viral[18] and parasitic infections[19] in CD40-deficient (CD40-KO) mice, suggesting that the CD40 signal compensates for the lack of RANK signaling in $CD4^+$ T cell priming by cDCs. Moreover, the role of the RANKL signal in the *in vivo* immune response was investigated in a spontaneous autoimmune disease model induced by the lack of IL-2[20]. The T cell-mediated intestinal inflammation in IL-2 deficient mice was significantly mitigated by the administration of osteoprotegerin (OPG)[21], a natural decoy receptor for RANKL[22,23]. Furthermore, the increase in the numbers of activated cDCs in the intestine of IL-2 deficient mice was significantly suppressed when the mice were treated with OPG. Thus, this study suggested that the RANKL-mediated survival of cDC promotes autoimmune inflammatory bowel disease *in vivo*. These studies support the idea that RANKL signaling activates the immune response by promoting the survival of cDCs, enhancing T-cell priming and activation. In contrast to these findings, the overexpression of OPG in rodents did not cause obvious changes in the innate or acquired immune response[24,25]. Overall, it is likely that the activation of the immune response by RANKL signaling occurs only in specific circumstances or redundantly with other cytokine signals, such as CD40.

Regulatory T cells

It is well recognized that the immune regulatory functions of DCs are dependent on the DC subtype and maturational stage[14]. Studies have been undertaken to investigate the role of RANKL signaling in the functions of DCs localized to the surface barriers of the body, such as the mucosal tissues and the skin. RANKL was shown to exhibit an immunosuppressive effect through these DCs (Figure 1). In the skin, the RANKL signal altered the function of the epidermal dendritic cells, increasing the number of Foxp3-positive regulatory T cells (Tregs)[26], a $CD4^+$ helper T cell subset critical for suppressing autoimmune responses and excess immune reactions in the body[27]. The expression of RANKL is upregulated in skin keratinocytes by ultraviolet light irradiation or inflammation[26] possibly mediated by the activity of prostaglandin E2[28]. Thus, stimulation of epidermal DCs with RANKL induces systemic immunosuppressive activity. In contrast to the effect of RANKL, the enforced expression of CD40L in keratinocytes induces severe autoimmune disease in the skin[26,29,30]. Interestingly, the autoimmune disease provoked by the enforced expression of CD40L is significantly suppressed by the enforced expression of RANKL in keratinocytes[26]. Thus, RANKL and CD40 signaling result in diametrically different immune responses in the skin. Moreover, in contrast to the autoimmune intestine disease model induced by IL-2 deficiency, which is described above[21], two studies suggest the immunosuppressive effects of RANKL signaling in the intestines[31,32]. Peyer's patches are lymphoid tissues located in the intestine that control the immune response to

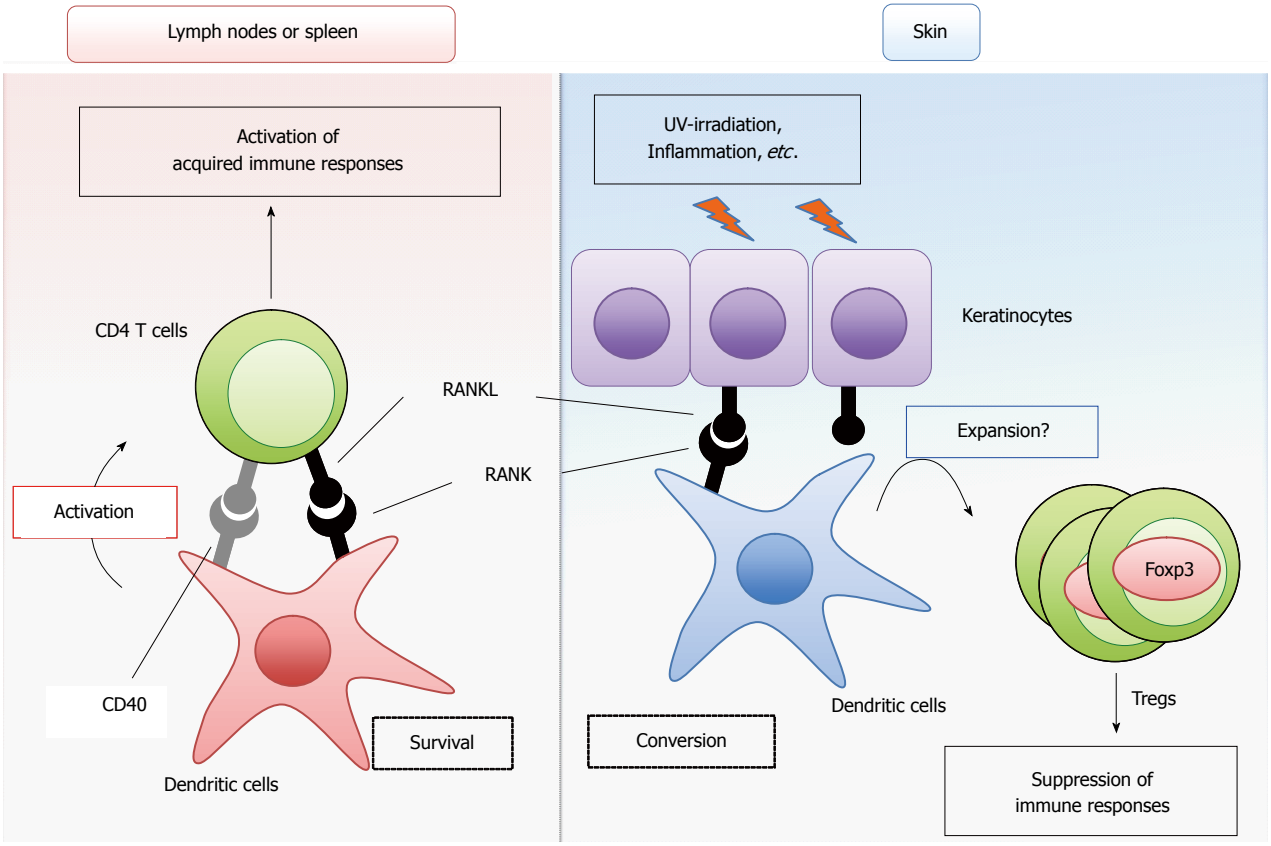

Figure 1 Modulation of dendritic cell functions by receptor activator of NF-κB ligand signaling. Receptor activator of NF-κB ligand (RANKL) signaling promotes the survival of conventional dendritic cells (cDCs) and ensures T cell priming and activation, thereby enhancing the acquired immune response (left). CD40 signaling may have a redundant role in this function of RANK signaling. In skin and, most likely, the intestines, RANKL-treated DCs maintain the number of Foxp3-positive regulatory T cells to suppress the immune response against self-antigens, food and commensal flora (right). In the skin, inflammation and ultraviolet (UV)-irradiation upregulate the expression of RANKL in keratinocytes.

foods or commensal bacteria. The stimulation of Peyer's patch-derived cDCs with RANKL increased the expression of IL-10[31], a cytokine with anti-inflammatory activity[33]. Consistently, the treatment of mice with RANKL enhanced the oral tolerance to an ovalbumin challenge[31]. In another study, a model of colitis induced by the transfer of the $CD4^+CD45RB^{high}$ T cell fraction into lymphopenic mice was utilized as a T cell-dependent autoimmune disease model[34]. In this model, colitis is suppressed by the simultaneous transfer of the $CD4^+CD25^+$ T cell fraction, which is enriched for $Foxp3^+$-regulatory T cells (Tregs)[34]. It was demonstrated that the administration of an anti-RANKL neutralizing antibody inhibits the suppression of colitis by the transfer of $CD4^+CD25^+$ T cells[32], suggesting that RANKL supports the function of the $CD4^+CD25^+$ regulatory T cells in the intestine. These data suggest that RANKL-mediated modulation of the surface barrier DCs results in the suppression of autoimmune responses and detrimental immune responses toward innocuous foreign antigens derived from foods or commensal bacteria in the intestines.

Lymphoid organogenesis

The lymphoid organs can be classified into primary lymphoid organs and secondary lymphoid organs. The primary lymphoid organs, the bone marrow and thymus, primarily provide the environment that is required for the differentiation and development of immune cells. The secondary lymphoid organs, including the lymph nodes, spleen and Peyer's patches, are tissues that trap antigens and initiate the acquired immune response. RANKL signaling is involved in the development and organization of these organs. Although the bone marrow is a primary lymphoid organ whose environment is regulated by RANKL, it is reviewed elsewhere in this issue[35] and is not further discussed here. Previous studies have revealed that RANKL- and RANK-KO mice lack lymph nodes[4-6]. Lymph nodes consist of several different types of cells, including T cells and B cells, DCs, reticular cells, stromal cells and specialized endothelial cells[36]. Communication between the lymphoid cells and the stromal cells is required for the formation and maintenance of the lymph node structure. Stromal cells express and secrete the chemokines CXCL13, CCL19 and CCL21 in the defined region of the lymph nodes, which are necessary for the accumulation and retention of B cells and T cells in distinct areas of the lymph nodes[37-39]. These lymphocytes in turn express cytokines that induce the expression and secretion of these chemokines from the stromal cells.

RANKL signaling controls the organogenesis of

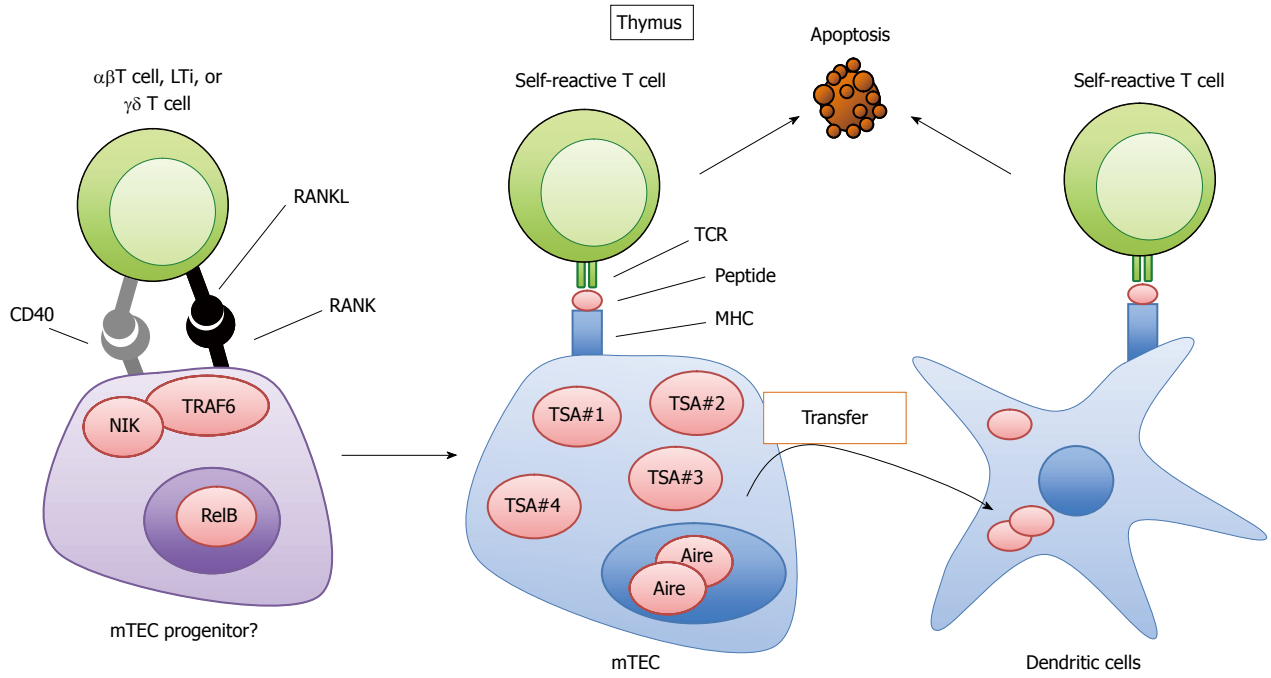

Figure 2 Regulation of the development and function of medullary thymic epithelial cells by receptor activator of NF-κB ligand signaling. Receptor activator of NF-κB ligand (RANKL) signaling mediates the development of mature medullary thymic epithelial cells (mTECs) expressing Autoimmune regulator (Aire) and a wide variety of tissue-specific antigens (TSAs) from the potential progenitors. RANKL is expressed in several types of cells, including αβ T cells, lymphoid tissue inducer cells (LTi) and a subset of γδ T cells. The signal transducers NF-κB inducing kinase (NIK) and TNF receptor-associated factor 6 (TRAF6) mediate RANK signaling, culminating in activation of the NF-κB transcription factor member RelB. mTECs present peptides are derived from TSAs to eliminate self-antigen reactive T cells by apoptosis. The TSAs can be transferred to dendritic cells by mechanisms that are not yet fully identified. The dendritic cells receiving TSAs also induce the apoptosis of self-reactive T cells in the thymus.

fetal lymph nodes. The early development of a lymph node begins with the movement of lymphoid tissue inducer cells (LTi), a unique cell subset derived from the fetal liver, into the region where the lymph nodes will develop[39-41]. The LTi interact with the resident stromal cells to initiate colonization and cluster formation, thereby forming lymph node anlages[39-41]. The TNF family members, lymphotoxinα (Ltα) and membrane-bound lymphotoxinβ (Ltβ), which are secreted by the LTi, are essential for lymph node organogenesis. Ltα and Ltβ form heterotrimers on the cell surface of LTi, which bind to the lymphotoxinβ receptor (LtβR) on the stromal cells. This interaction induces the expressions of chemokines and adhesion molecules by the stromal cells to attract and retain LTi. A complex pattern of RANKL expression has been detected during the process of early lymph node development[42,43]. RANKL is initially expressed in the LTi[42,43], which is independent of LtβR signaling[42]. Subsequently, lymphoid tissue organizer cells derived from mesenchymal stromal cells initiate the expression of RANKL in a LtβR signal-dependent manner[42]. A deficiency in RANKL signaling results in a severe reduction in the number of LTi[5], suggesting that the requirement for RANKL signaling begins during the initial LTi colonization process of early development. Interestingly, whereas RANKL- and RANK-KO null mice lack lymph nodes, the inhibition of RANKL signaling by the overexpression of its decoy receptor OPG did not impair the development of lymph nodes[24,25], implying that very small quantities and the temporary expression of RANKL are sufficient for the development of lymph nodes. Notably, whereas LtβR signaling is also essential for the development of Peyer's patches, which are mucosal lymphoid organs located in the intestines, RANKL-KO mice contain Peyer's patches[4,5]. Consistently, TRAF6, a critical signal transducer of RANK signaling, is required for lymph node development but not for the development of Peyer's patches[44,45]. Thus, the RANKL-RANK-TRAF6 axis is dispensable for the formation of Peyer's patches. Moreover, the formation of the splenic architecture is regulated by TNF family signals and chemokine signals[46]. However, the splenic architecture is not practically impaired by the absence of RANK signaling[4,5].

Interestingly, although RANKL is not essential for the formation of Peyer's patches, the expression of RANKL was detected in the stromal cells of Peyer's patches and other lymphoid tissues of the intestine[47]. Moreover, the Peyer's patches of RANKL-KO mice are smaller than in control mice[4,5]. These observations imply that RANKL signaling may contribute to the formation of the microarchitecture of Peyer's patches. Indeed, a recent study has uncovered a role for the RANK signal in the development of M cells[48], a specialized subset of epithelial cells found in mucosal lymphoid organs, such as Peyer's patches and isolated lymphoid follicles[49]. M cells are localized to the epithelial cell layers that cover these tissues and they transcytose particulate foreign antigens and mi-

croorganisms from the lumen. This process is critical for controlling the immune response against pathogens and commensal flora[50]. RANKL-KO mice possessed fewer M cells in Peyer's patches[48]. Moreover, the administration of an anti-RANKL neutralizing antibody reduced the number of M cells[48]. Thus, this study supported a novel role of RANKL signaling in the initiation of M cell development.

THYMIC EPITHELIAL CELLS AND T CELL SELECTION

Thymic epithelial cells (TECs) are essential for the development and selection of T cells in the thymus[51,52]. Several recent studies have revealed that RANKL signaling promotes the development of a subset of TECs that are essential for preventing autoimmunity by eliminating self-reactive T cells in the thymus[53-55]. We briefly describe the fundamental knowledge regarding T cell selection and development in the thymus and its correlation with TECs. Then, we summarize the contribution of RANKL signaling to the development of TECs and to preventing autoimmunity.

Avidity model

A large portion of the T cells in the body develop in the thymus and developed T cells express widely diverse "repertoires" of T cell antigen receptors (TCR) with different antigen specificities. After the TCR is expressed on the cell surface, the T cell repertoires are shaped according to two aspects; the first is to recognize self-major histocompatibility complex (MHC) molecules and the second is to be unresponsive to self-antigens[56]. The achievement of this selectivity is explained by the so-called "avidity (affinity)" model[57,58]. The basic concept of this model is that the fate of each T cell is dependent on the avidity between its T cell antigen receptor (TCR) and complexes of self-antigen peptides and MHC molecules, which are presented by antigen-presenting cells in the thymus. T cells recognizing the self antigen-MHC complex with very low or high avidity through their TCRs undergo apoptosis and only the T cells recognizing the self antigen-MHC complex with "moderate" affinity can survive. As result, T cells incapable of binding to self-MHC and T cells responsive to self antigens in other tissues would be eliminated by this mechanism. Thus, this selection mechanism permits the generation of T cell repertoires that recognize foreign antigens that bind to self-MHC with high affinity.

T cell selection

T cell selection is closely related to the development of T cells in the thymus. The thymus is anatomically divided into the cortex and the medulla[59,60]. Different developmental and selection processes occur in each region[59,60]. First, T lymphoid progenitor cells from the bone marrow develop into CD4 and CD8 double-positive immature T cells ($CD4^+CD8^+$ T cells) in the cortex. Subsequently, the $CD4^+CD8^+$ T cells interact with epithelial cells in the cortex (cTECs; cortical thymic epithelial cells), which are antigen-presenting cells expressing MHC molecules. Through this interaction, the $CD4^+CD8^+$ T cells that recognize the complex of self-antigen and MHC molecules presented by cTEC survive and successfully differentiate into CD4 or CD8 single-positive (CD4SP or CD8SP) T cells. The CD4SP or CD8SP T cells move to the medulla and are further scanned by the medullary thymic epithelial cells (mTECs). The mTECs have unusual properties with regards to gene expression; mTECs ectopically express a wide variety of self-antigens that are normally expressed in a tissue-restricted manner (TSAs, for example, insulin or caseins) (Figure 2)[61]. The ectopic expression of TSAs is in part regulated by the nuclear protein Aire (autoimmune regulator)[62,63], which suppresses the onset of autoimmune disease in humans and mice[63,64]. mTECs present TSAs to the medullary T cells directly or indirectly through the thymic dendritic cells (Figure 2)[65-67]. Those T cell repertoires that recognize TSA peptide with high avidity are eliminated (Figure 2); otherwise, they would initiate an immune response to the TSAs expressed in their cognate organs, potentially causing autoimmune disease. In fact, several lines of evidence have indicated that the dysregulation of this mechanism promotes autoimmune disease[63,68-71].

RANKL signaling in mTEC development

It is currently accepted that both mTECs and cTECs differentiate from common progenitor cells[72,73]. Moreover, evidence has suggested the presence of an mTEC progenitor[74]. However, the details of the developmental process from common progenitor to mature mTECs and cTECs are largely unknown. Several studies have revealed a role for RANKL signaling in the development of mature mTECs expressing Aire and TSAs[53-55] (Figure 2). The expression of RANK was detected in mTECs in the adult thymus[54,55] and in fetal thymic stroma organ cultures[54], which plausibly contain mTEC progenitors[53]. RANKL- and RANK-KO mice exhibited a partial defect in the development of mature mTECs[53-55]. The quantity of Aire-positive in mature mTECs is preferentially reduced by the lack of RANKL and RANK. Whereas mTEC levels are significantly reduced by the absence of RANK signals, a population of mature mTECs still exists. However, TRAF6 deficiency results in a severe defect in mTEC development compared to that in RANKL and RANK-deficient mice[68]. This difference can be explained by CD40-mediated compensation for RANK during TEC development, which is shown by the complete loss of mTECs in RANKL and CD40 double-deficient mice. Thus, RANK and CD40 cooperatively signal to promote the development of mTECs, which appears to be similar to the cooperation between RANK and CD40 in modulating the functions of cDCs[18].

RANKL signaling suppresses autoimmunity

The requirement for RANK signaling for the prevention

of autoimmunity has also been tested. The transplantation of RANK-deficient fetal thymic stroma into nude mice results in inflammatory cell infiltration accompanied by the production of autoantibodies in the sera[53]. These data suggest that the lack of RANK in the thymic stromal cells is sufficient to induce autoimmunity. Another study demonstrated that the transfer of splenocytes from RANKL-KO mice into nude mice provokes mild autoimmunity[54]. Moreover, a much more severe autoimmune response was observed when splenocytes from RANKL and CD40 double-deficient mice were transferred into nude mice[54], which appeared to be correlated with the severe impairment in mTEC development in these doubly deficient mice. Consistently, mutant mouse lines lacking downstream molecules in the RANK signaling pathway (e.g., RelB-deficient mice or TRAF6-deficient mice) displayed severe phenotypes[68,75,76].

Thymic cells providing RANKL

Several types of cells have been reported to express RANKL in the thymus. Interestingly, a previous study has revealed that LTi are localized to the embryonic thymus and provide RANKL for the embryonic development of Aire-expressing mTECs[53]. Moreover, the T cells that had newly differentiated from $CD4^+CD8^+$ T cells into CD4SP T cells were found to express high levels of RANKL[55]. A recent study has revealed that dendritic epidermal T cells (DETC), a subset of γδ T cells[77], express RANKL in the embryonic thymus[78]. In this study, it was also shown that RANKL signaling induced the expression of the immunoglobulin superfamily member Skint-1, which is involved in the selection of the DETC[79] that contribute to immune defense in the skin[77]. Thus, this study uncovered a novel connection between mTEC development and γδ T cell development in the fetal thymus mediated by the RANKL and RANK interaction.

Expression of RANK

The molecular mechanism underlying the expression of RANK in the mTEC progenitors remains unclear. A recent study has suggested that lymphotoxin beta receptor (LtβR) signaling is involved in the expression of RANK in immature mTECs[80]. This study suggested that RANK expression in TECs is under the control of LtβR signaling. However, it still remains unclear whether the LtβR signal upregulates the expression of RANK in individual TECs or promotes the proliferation and/or survival of RANK-expressing mTECs.

SIGNALING PATHWAY INDUCED BY RANKL SIGNALING IN TEC

The interaction between RANKL and RANK induces activation of the NF-κB and the Mitogen-Activated Protein Kinase (MAPK) pathways[7]. During mTEC development, the NF-κB activation pathway appears to play a major role. Two major NF-κB activation pathways, the classical pathway and the non-classical pathway, are currently known[81]. The classical pathway induces the translocation of the RelA or c-Rel complex into the nucleus through the activation of the IκB kinase (IKK) complex containing IKKβ and NEMO. The activation of the non-classical pathway results in the translocation of RelB into the nucleus and requires the phosphorylation of IKKα by NF-κB inducing kinase (NIK). RANK signaling is capable of activating both pathways[82]. Interestingly, whereas both NF-kB activation pathways appear to be activated during mTEC and osteoclast development induced by RANKL, the contribution of each NF-κB pathway appears to differ between mTEC development and osteoclast differentiation. For instance, RelB-deficient (RelB-KO) mice, NIK-deficient mice and *aly/aly* mice, the last of which have a dysfunctional mutation in the NIK gene, exhibited normal or very mildly impaired osteoclastogenesis[82-86] and this pathway appears to be critical only in pathological osteolysis[82,83]. However, mTEC development was completely abolished in *aly/aly* and RelB-deficient mice[75,76,87]. Moreover, IKKα-deficient fetal thymic stroma did not differentiate into mature mTECs when transplanted onto the kidney[88]. In an *in vitro* study, RANKL-dependent maturation of mTEC was not detected in fetal thymic stroma prepared from *aly/aly* mice[54]. Moreover, RANKL signaling induces the nuclear localization of RelB in mature mTECs in fetal thymic stroma[54]. Thus, these data suggest that, in contrast to its involvement in osteoclast development, the non-classical NF-κB activation pathway is essential for the RANKL-dependent development of mTECs. The contribution of the classical NF-κB pathway to mTEC development has been suggested by the finding that TRAF6 is essential for the development of mTECs[54,68] as TRAF6 activates the classical NF-κB pathway and is dispensable for the non-classical NF-κB pathway[89]. Whereas c-Fos and NF-ATc1 are critical regulators of RANKL-mediated osteoclast development, the involvement of these genes in mTEC development has not been reported. These data suggest that RANKL signaling during mTEC development activates signal transduction pathways that are distinct from those activated during osteoclast development.

CONCLUDING REMARKS

A humanized anti-RANKL antibody has been approved for the treatment of osteoporosis in postmenopausal women and cancer bone metastasis. Detailed studies in mouse models have clearly demonstrated the involvement of RANKL signaling in the functions of immune regulatory cells, such as dendritic cells, M cells and mTECs. Notably, the functions of dendritic cells and the maintenance of M cell numbers were impaired by the inhibition of RANKL signaling in adult mice. These results may be informative in applications of the anti-RANKL antibody for human treatments. Moreover, these relatively new findings could open the possibility of utilizing the anti-RANKL antibody for other applications by regulating the immune response.

REFERENCES

1 **Wong BR**, Rho J, Arron J, Robinson E, Orlinick J, Chao M, Kalachikov S, Cayani E, Bartlett FS, Frankel WN, Lee SY, Choi Y. TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. *J Biol Chem* 1997; **272**: 25190-25194

2 **Anderson DM**, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 1997; **390**: 175-179

3 **Yasuda H**, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci USA* 1998; **95**: 3597-3602

4 **Kong YY**, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 1999; **397**: 315-323

5 **Kim D**, Mebius RE, MacMicking JD, Jung S, Cupedo T, Castellanos Y, Rho J, Wong BR, Josien R, Kim N, Rennert PD, Choi Y. Regulation of peripheral lymph node genesis by the tumor necrosis factor family member TRANCE. *J Exp Med* 2000; **192**: 1467-1478

6 **Dougall WC**, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J. RANK is essential for osteoclast and lymph node development. *Genes Dev* 1999; **13**: 2412-2424

7 **Darnay BG**, Haridas V, Ni J, Moore PA, Aggarwal BB. Characterization of the intracellular domain of receptor activator of NF-kappaB (RANK). Interaction with tumor necrosis factor receptor-associated factors and activation of NF-kappab and c-Jun N-terminal kinase. *J Biol Chem* 1998; **273**: 20551-20555

8 **Wong BR**, Josien R, Lee SY, Vologodskaia M, Steinman RM, Choi Y. The TRAF family of signal transducers mediates NF-kappaB activation by the TRANCE receptor. *J Biol Chem* 1998; **273**: 28355-28359

9 **Galibert L**, Tometsko ME, Anderson DM, Cosman D, Dougall WC. The involvement of multiple tumor necrosis factor receptor (TNFR)-associated factors in the signaling mechanisms of receptor activator of NF-kappaB, a member of the TNFR superfamily. *J Biol Chem* 1998; **273**: 34120-34127

10 **Darnay BG**, Besse A, Poblenz AT, Lamothe B, Jacoby JJ. TRAFs in RANK signaling. *Adv Exp Med Biol* 2007; **597**: 152-159

11 **Bishop GA**, Moore CR, Xie P, Stunz LL, Kraus ZJ. TRAF proteins in CD40 signaling. *Adv Exp Med Biol* 2007; **597**: 131-151

12 **Quezada SA**, Jarvinen LZ, Lind EF, Noelle RJ. CD40/CD154 interactions at the interface of tolerance and immunity. *Annu Rev Immunol* 2004; **22**: 307-328

13 **Ma DY**, Clark EA. The role of CD40 and CD154/CD40L in dendritic cells. *Semin Immunol* 2009; **21**: 265-272

14 **Belz GT**, Nutt SL. Transcriptional programming of the dendritic cell network. *Nat Rev Immunol* 2012; **12**: 101-113

15 **Josien R**, Wong BR, Li HL, Steinman RM, Choi Y. TRANCE, a TNF family member, is differentially expressed on T cell subsets and induces cytokine production in dendritic cells. *J Immunol* 1999; **162**: 2562-2568

16 **Wong BR**, Josien R, Lee SY, Sauter B, Li HL, Steinman RM, Choi Y. TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. *J Exp Med* 1997; **186**: 2075-2080

17 **Josien R**, Li HL, Ingulli E, Sarma S, Wong BR, Vologodskaia M, Steinman RM, Choi Y. TRANCE, a tumor necrosis factor family member, enhances the longevity and adjuvant properties of dendritic cells in vivo. *J Exp Med* 2000; **191**: 495-502

18 **Bachmann MF**, Wong BR, Josien R, Steinman RM, Oxenius A, Choi Y. TRANCE, a tumor necrosis factor family member critical for CD40 ligand-independent T helper cell activation. *J Exp Med* 1999; **189**: 1025-1031

19 **Padigel UM**, Kim N, Choi Y, Farrell JP. TRANCE-RANK costimulation is required for IL-12 production and the initiation of a Th1-type response to Leishmania major infection in CD40L-deficient mice. *J Immunol* 2003; **171**: 5437-5441

20 **Schimpl A**, Berberich I, Kneitz B, Krämer S, Santner-Nanan B, Wagner S, Wolf M, Hünig T. IL-2 and autoimmune disease. *Cytokine Growth Factor Rev* 2002; **13**: 369-378

21 **Ashcroft AJ**, Cruickshank SM, Croucher PI, Perry MJ, Rollinson S, Lippitt JM, Child JA, Dunstan C, Felsburg PJ, Morgan GJ, Carding SR. Colonic dendritic cells, intestinal inflammation, and T cell-mediated bone destruction are modulated by recombinant osteoprotegerin. *Immunity* 2003; **19**: 849-861

22 **Bucay N**, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998; **12**: 1260-1268

23 **Mizuno A**, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, Sato Y, Nakagawa N, Yasuda H, Mochizuki S, Gomibuchi T, Yano K, Shima N, Washida N, Tsuda E, Morinaga T, Higashio K, Ozawa H. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. *Biochem Biophys Res Commun* 1998; **247**: 610-615

24 **Simonet WS**, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309-319

25 **Stolina M**, Dwyer D, Ominsky MS, Corbin T, Van G, Bolon B, Sarosi I, McCabe J, Zack DJ, Kostenuik P. Continuous RANKL inhibition in osteoprotegerin transgenic mice and rats suppresses bone resorption without impairing lymph organogenesis or functional immune responses. *J Immunol* 2007; **179**: 7497-7505

26 **Loser K**, Mehling A, Loeser S, Apelt J, Kuhn A, Grabbe S, Schwarz T, Penninger JM, Beissert S. Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. *Nat Med* 2006; **12**: 1372-1379

27 **Josefowicz SZ**, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol* 2012; **30**: 531-564

28 **Soontrapa K**, Honda T, Sakata D, Yao C, Hirata T, Hori S, Matsuoka T, Kita Y, Shimizu T, Kabashima K, Narumiya S. Prostaglandin E2-prostaglandin E receptor subtype 4 (EP4) signaling mediates UV irradiation-induced systemic immunosuppression. *Proc Natl Acad Sci USA* 2011; **108**: 6668-6673

29 **Clegg CH**, Rulffes JT, Haugen HS, Hoggatt IH, Aruffo A, Durham SK, Farr AG, Hollenbaugh D. Thymus dysfunction and chronic inflammatory disease in gp39 transgenic mice. *Int Immunol* 1997; **9**: 1111-1122

30 **Mehling A**, Loser K, Varga G, Metze D, Luger TA, Schwarz T, Grabbe S, Beissert S. Overexpression of CD40 ligand in murine epidermis results in chronic skin inflammation and systemic autoimmunity. *J Exp Med* 2001; **194**: 615-628

31 **Williamson E**, Bilsborough JM, Viney JL. Regulation of mucosal dendritic cell function by receptor activator of NF-

kappa B (RANK)/RANK ligand interactions: impact on tolerance induction. *J Immunol* 2002; **169**: 3606-3612
32 **Totsuka T**, Kanai T, Nemoto Y, Tomita T, Okamoto R, Tsuchiya K, Nakamura T, Sakamoto N, Akiba H, Okumura K, Yagita H, Watanabe M. RANK-RANKL signaling pathway is critically involved in the function of CD4+CD25+ regulatory T cells in chronic colitis. *J Immunol* 2009; **182**: 6079-6087
33 **Saraiva M**, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 2010; **10**: 170-181
34 **Coombes JL**, Robinson NJ, Maloy KJ, Uhlig HH, Powrie F. Regulatory T cells and intestinal homeostasis. *Immunol Rev* 2005; **204**: 184-194
35 **Kuroda Y**, Matsuo K. Molecular mechanisms of triggering, amplifying and targeting RANK signaling in osteoclasts. *World J Orthop* 2012; In press
36 **Ruddle NH**, Akirav EM. Secondary lymphoid organs: responding to genetic and environmental cues in ontogeny and the immune response. *J Immunol* 2009; **183**: 2205-2212
37 **Cyster JG**, Ansel KM, Reif K, Ekland EH, Hyman PL, Tang HL, Luther SA, Ngo VN. Follicular stromal cells and lymphocyte homing to follicles. *Immunol Rev* 2000; **176**: 181-193
38 **Drayton DL**, Liao S, Mounzer RH, Ruddle NH. Lymphoid organ development: from ontogeny to neogenesis. *Nat Immunol* 2006; **7**: 344-353
39 **Mebius RE**. Organogenesis of lymphoid tissues. *Nat Rev Immunol* 2003; **3**: 292-303
40 **Nishikawa S**, Honda K, Vieira P, Yoshida H. Organogenesis of peripheral lymphoid organs. *Immunol Rev* 2003; **195**: 72-80
41 **Finke D**. Fate and function of lymphoid tissue inducer cells. *Curr Opin Immunol* 2005; **17**: 144-150
42 **Vondenhoff MF**, Greuter M, Goverse G, Elewaut D, Dewint P, Ware CF, Hoorweg K, Kraal G, Mebius RE. LTbetaR signaling induces cytokine expression and up-regulates lymphangiogenic factors in lymph node anlagen. *J Immunol* 2009; **182**: 5439-5445
43 **Sugiyama M**, Nakato G, Jinnohara T, Akiba H, Okumura K, Ohno H, Yoshida H. Expression pattern changes and function of RANKL during mouse lymph node microarchitecture development. *Int Immunol* 2012; **24**: 369-378
44 **Naito A**, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, Nakao K, Nakamura K, Katsuki M, Yamamoto T, Inoue J. Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. *Genes Cells* 1999; **4**: 353-362
45 **Lomaga MA**, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der Heiden A, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV, Mak TW. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev* 1999; **13**: 1015-1024
46 **Mebius RE**, Kraal G. Structure and function of the spleen. *Nat Rev Immunol* 2005; **5**: 606-616
47 **Taylor RT**, Patel SR, Lin E, Butler BR, Lake JG, Newberry RD, Williams IR. Lymphotoxin-independent expression of TNF-related activation-induced cytokine by stromal cells in cryptopatches, isolated lymphoid follicles, and Peyer's patches. *J Immunol* 2007; **178**: 5659-5667
48 **Knoop KA**, Kumar N, Butler BR, Sakthivel SK, Taylor RT, Nochi T, Akiba H, Yagita H, Kiyono H, Williams IR. RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epithelium. *J Immunol* 2009; **183**: 5738-5747
49 **Kraehenbuhl JP**, Neutra MR. Epithelial M cells: differentiation and function. *Annu Rev Cell Dev Biol* 2000; **16**: 301-332
50 **Iweala OI**, Nagler CR. Immune privilege in the gut: the establishment and maintenance of non-responsiveness to dietary antigens and commensal flora. *Immunol Rev* 2006; **213**: 82-100
51 **Klein L**, Hinterberger M, Wirnsberger G, Kyewski B. Antigen presentation in the thymus for positive selection and central tolerance induction. *Nat Rev Immunol* 2009; **9**: 833-844
52 **Anderson G**, Takahama Y. Thymic epithelial cells: working class heroes for T cell development and repertoire selection. *Trends Immunol* 2012; **33**: 256-263
53 **Rossi SW**, Kim MY, Leibbrandt A, Parnell SM, Jenkinson WE, Glanville SH, McConnell FM, Scott HS, Penninger JM, Jenkinson EJ, Lane PJ, Anderson G. RANK signals from CD4(+)3(-) inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. *J Exp Med* 2007; **204**: 1267-1272
54 **Akiyama T**, Shimo Y, Yanai H, Qin J, Ohshima D, Maruyama Y, Asaumi Y, Kitazawa J, Takayanagi H, Penninger JM, Matsumoto M, Nitta T, Takahama Y, Inoue J. The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. *Immunity* 2008; **29**: 423-437
55 **Hikosaka Y**, Nitta T, Ohigashi I, Yano K, Ishimaru N, Hayashi Y, Matsumoto M, Matsuo K, Penninger JM, Takayanagi H, Yokota Y, Yamada H, Yoshikai Y, Inoue J, Akiyama T, Takahama Y. The cytokine RANKL produced by positively selected thymocytes fosters medullary thymic epithelial cells that express autoimmune regulator. *Immunity* 2008; **29**: 438-450
56 **Starr TK**, Jameson SC, Hogquist KA. Positive and negative selection of T cells. *Annu Rev Immunol* 2003; **21**: 139-176
57 **Morris GP**, Allen PM. How the TCR balances sensitivity and specificity for the recognition of self and pathogens. *Nat Immunol* 2012; **13**: 121-128
58 **Hogquist KA**, Baldwin TA, Jameson SC. Central tolerance: learning self-control in the thymus. *Nat Rev Immunol* 2005; **5**: 772-782
59 **Gill J**, Malin M, Sutherland J, Gray D, Hollander G, Boyd R. Thymic generation and regeneration. *Immunol Rev* 2003; **195**: 28-50
60 **Takahama Y**. Journey through the thymus: stromal guides for T-cell development and selection. *Nat Rev Immunol* 2006; **6**: 127-135
61 **Kyewski B**, Klein L. A central role for central tolerance. *Annu Rev Immunol* 2006; **24**: 571-606
62 **Mathis D**, Benoist C. Aire. *Annu Rev Immunol* 2009; **27**: 287-312
63 **Anderson MS**, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002; **298**: 1395-1401
64 **Michels AW**, Gottlieb PA. Autoimmune polyglandular syndromes. *Nat Rev Endocrinol* 2010; **6**: 270-277
65 **Gallegos AM**, Bevan MJ. Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation. *J Exp Med* 2004; **200**: 1039-1049
66 **Koble C**, Kyewski B. The thymic medulla: a unique microenvironment for intercellular self-antigen transfer. *J Exp Med* 2009; **206**: 1505-1513
67 **Hinterberger M**, Aichinger M, Prazeres da Costa O, Voehringer D, Hoffmann R, Klein L. Autonomous role of medullary thymic epithelial cells in central CD4(+) T cell tolerance. *Nat Immunol* 2010; **11**: 512-519
68 **Akiyama T**, Maeda S, Yamane S, Ogino K, Kasai M, Kajiura F, Matsumoto M, Inoue J. Dependence of self-tolerance on TRAF6-directed development of thymic stroma. *Science* 2005; **308**: 248-251
69 **DeVoss J**, Hou Y, Johannes K, Lu W, Liou GI, Rinn J, Chang H, Caspi RR, Fong L, Anderson MS. Spontaneous autoimmunity prevented by thymic expression of a single self-antigen. *J Exp Med* 2006; **203**: 2727-2735
70 **Giraud M**, Taubert R, Vandiedonck C, Ke X, Lévi-Strauss M, Pagani F, Baralle FE, Eymard B, Tranchant C, Gajdos P, Vincent A, Willcox N, Beeson D, Kyewski B, Garchon HJ. An

IRF8-binding promoter variant and AIRE control CHRNA1 promiscuous expression in thymus. *Nature* 2007; **448**: 934-937
71 **Fan Y**, Rudert WA, Grupillo M, He J, Sisino G, Trucco M. Thymus-specific deletion of insulin induces autoimmune diabetes. *EMBO J* 2009; **28**: 2812-2824
72 **Rossi SW**, Jenkinson WE, Anderson G, Jenkinson EJ. Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium. *Nature* 2006; **441**: 988-991
73 **Bleul CC**, Corbeaux T, Reuter A, Fisch P, Mönting JS, Boehm T. Formation of a functional thymus initiated by a postnatal epithelial progenitor cell. *Nature* 2006; **441**: 992-996
74 **Rodewald HR**, Paul S, Haller C, Bluethmann H, Blum C. Thymus medulla consisting of epithelial islets each derived from a single progenitor. *Nature* 2001; **414**: 763-768
75 **Burkly L**, Hession C, Ogata L, Reilly C, Marconi LA, Olson D, Tizard R, Cate R, Lo D. Expression of relB is required for the development of thymic medulla and dendritic cells. *Nature* 1995; **373**: 531-536
76 **Weih F**, Carrasco D, Durham SK, Barton DS, Rizzo CA, Ryseck RP, Lira SA, Bravo R. Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF-kappa B/Rel family. *Cell* 1995; **80**: 331-340
77 **Macleod AS**, Havran WL. Functions of skin-resident γδ T cells. *Cell Mol Life Sci* 2011; **68**: 2399-2408
78 **Roberts NA**, White AJ, Jenkinson WE, Turchinovich G, Nakamura K, Withers DR, McConnell FM, Desanti GE, Benezech C, Parnell SM, Cunningham AF, Paolino M, Penninger JM, Simon AK, Nitta T, Ohigashi I, Takahama Y, Caamano JH, Hayday AC, Lane PJ, Jenkinson EJ, Anderson G. Rank signaling links the development of invariant γδ T cell progenitors and Aire(+) medullary epithelium. *Immunity* 2012; **36**: 427-437
79 **Boyden LM**, Lewis JM, Barbee SD, Bas A, Girardi M, Hayday AC, Tigelaar RE, Lifton RP. Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal gammadelta T cells. *Nat Genet* 2008; **40**: 656-662
80 **Mouri Y**, Yano M, Shinzawa M, Shimo Y, Hirota F, Nishikawa Y, Nii T, Kiyonari H, Abe T, Uehara H, Izumi K, Tamada K, Chen L, Penninger JM, Inoue J, Akiyama T, Matsumoto M. Lymphotoxin signal promotes thymic organogenesis by eliciting RANK expression in the embryonic thymic stroma. *J Immunol* 2011; **186**: 5047-5057
81 **Oeckinghaus A**, Hayden MS, Ghosh S. Crosstalk in NF-κB signaling pathways. *Nat Immunol* 2011; **12**: 695-708
82 **Novack DV**, Yin L, Hagen-Stapleton A, Schreiber RD, Goeddel DV, Ross FP, Teitelbaum SL. The IkappaB function of NF-kappaB2 p100 controls stimulated osteoclastogenesis. *J Exp Med* 2003; **198**: 771-781
83 **Vaira S**, Johnson T, Hirbe AC, Alhawagri M, Anwisye I, Sammut B, O'Neal J, Zou W, Weilbaecher KN, Faccio R, Novack DV. RelB is the NF-kappaB subunit downstream of NIK responsible for osteoclast differentiation. *Proc Natl Acad Sci USA* 2008; **105**: 3897-3902
84 **Maruyama T**, Fukushima H, Nakao K, Shin M, Yasuda H, Weih F, Doi T, Aoki K, Alles N, Ohya K, Hosokawa R, Jimi E. Processing of the NF-kappa B2 precursor p100 to p52 is critical for RANKL-induced osteoclast differentiation. *J Bone Miner Res* 2010; **25**: 1058-1067
85 **Soysa NS**, Alles N, Weih D, Lovas A, Mian AH, Shimokawa H, Yasuda H, Weih F, Jimi E, Ohya K, Aoki K. The pivotal role of the alternative NF-kappaB pathway in maintenance of basal bone homeostasis and osteoclastogenesis. *J Bone Miner Res* 2010; **25**: 809-818
86 **Shinzawa M**, Maruyama Y, Qin J, Akiyama N, Miyauchi M, Yanai H, Takami M, Inoue J, Akiyama T. Splenic extramedullary hemopoiesis caused by a dysfunctional mutation in the NF-κB-inducing kinase gene. *Biochem Biophys Res Commun* 2011; **414**: 773-778
87 **Kajiura F**, Sun S, Nomura T, Izumi K, Ueno T, Bando Y, Kuroda N, Han H, Li Y, Matsushima A, Takahama Y, Sakaguchi S, Mitani T, Matsumoto M. NF-kappa B-inducing kinase establishes self-tolerance in a thymic stroma-dependent manner. *J Immunol* 2004; **172**: 2067-2075
88 **Kinoshita D**, Hirota F, Kaisho T, Kasai M, Izumi K, Bando Y, Mouri Y, Matsushima A, Niki S, Han H, Oshikawa K, Kuroda N, Maegawa M, Irahara M, Takeda K, Akira S, Matsumoto M. Essential role of IkappaB kinase alpha in thymic organogenesis required for the establishment of self-tolerance. *J Immunol* 2006; **176**: 3995-4002
89 **Qin J**, Konno H, Ohshima D, Yanai H, Motegi H, Shimo Y, Hirota F, Matsumoto M, Takaki S, Inoue J, Akiyama T. Developmental stage-dependent collaboration between the TNF receptor-associated factor 6 and lymphotoxin pathways for B cell follicle organization in secondary lymphoid organs. *J Immunol* 2007; **179**: 6799-6807

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BRIEF ARTICLE

Injury patterns of seniors in traffic accidents: A technical and medical analysis

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Abstract

AIM: To investigate the actual injury situation of seniors in traffic accidents and to evaluate the different injury patterns.

METHODS: Injury data, environmental circumstances and crash circumstances of accidents were collected shortly after the accident event at the scene. With these data, a technical and medical analysis was performed, including Injury Severity Score, Abbreviated Injury Scale and Maximum Abbreviated Injury Scale. The method of data collection is named the German In-Depth Accident Study and can be seen as representative.

RESULTS: A total of 4430 injured seniors in traffic accidents were evaluated. The incidence of sustaining severe injuries to extremities, head and maxillofacial region was significantly higher in the group of elderly people compared to a younger age ($P < 0.05$). The number of accident-related injuries was higher in the group of seniors compared to other groups.

CONCLUSION: Seniors are more likely to be involved in traffic injuries and to sustain serious to severe injuries compared to other groups.

Key words: Traffic accidents; Seniors; Head injury; Injury severity score; Abbreviated injury scale

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INTRODUCTION

Due to the rising mean age of the German population, traffic accidents involving older people are observed more and more often. Furthermore, previous investigations have shown that geriatric trauma patients are more often associated with a higher level of mortality, morbid-

ity and frequency of more severe injuries[1-4]. There is not only an increase in age, but also in motorization in our society. A growing number of elderly people are taking part daily in traffic scenes, so the number and the severity of accidents involving older pedestrians[1,5,6], bicyclists or older people using motorized vehicles is increasing[2,6-9]. Furthermore, older people are less resistant to trauma due to co morbidities, e.g., osteoporosis[4]. Additionally, with the increasing mean age comes a decline in capabilities which impairs cognitive, sensory and psychomotor skills that are needed to avoid traffic accidents[4,7,10,11]. Also, older people are responsible for increasing costs in public health care programs due to prolonged post traumatic hospital stays[12].

In our retrospective study based on the accident data from the German In-Depth Accident Study (GIDAS), we hypothesized that older people are involved in traffic accidents by a higher rate[13], sustain more severe injuries by lower velocity trauma than younger patients[14], sustain more severe injuries, especially to the head and extremities[15], and even cause more accidents than other groups[7]. GIDAS is the largest in-depth accident study in Germany. The data collected in the GIDAS project is extensive. We used technical in-depth crash investigations in combination with medical data analysis to evaluate the injury situation of the elderly in traffic accidents.

MATERIALS AND METHODS

Technical in-depth crash investigations in combination with medical data analysis were performed by specially trained documentation personnel from our local in-depth accident research unit as a part of the trauma department of the Medical School, Hannover. This documentation crew is notified by police dispatchers immediately after an accident and arrives on the scene, often simultaneously with the rescue personnel. Investigation of the crash and clinical injury documentation is then performed on site. This case report is then completed at the hospital, where all of the injured victims are taken, with proper documentation of X-ray examination, injury type and severity. The monitoring includes demographic data, the area of collision, environmental circumstances and injury patterns. Furthermore, it includes specific outcome and severity scores, such as Abbreviated Injury Scale (AIS), Injury Severity Score (ISS) and Maximum Abbreviated Injury Scale (MAIS)[16], incidence of serious or severe multiple injuries (polytrauma, AIS 3+ in two different body regions of the patient admitted to the hospital), incidence of serious (MAIS, 2-4) or severe injuries (MAIS, 5-6), and mortality.

ISS is a well-known medical score to assess trauma severity. AIS is a severity scoring system that classifies injuries in each body region. Classification ranges from minor to maximum (1-6), indicating the relative severity of each body region. MAIS indicates the maximum AI-Scale of a trauma victim. Different technical measurement techniques are performed to document the exact crash constellation at the scene. Reconstructions of the crash and vehicle site, as well as vehicle movements and human behaviors, are then performed for later technical analysis options. Slide and skid marks of objects and victims and any kind of deformation of involved vehicles or objects are also measured and these data are included in the crash analysis. The amount of injuries is directly related to the travelling speed at the time of the accident. Collisions between pedestrians or bicyclists with cars are measured by the collision speed of the car.

For this retrospective study, cases from 1999-2011 from the local traffic research unit were analyzed for the involvement of injured seniors and followed by demographic data, injury scores, incidence of death, collision speed and collision type. For this study, prevalence from the preselected patient population was investigated.

Statistical analysis

For statistical analysis of the correlation between crash circumstances with injury severity (AIS/MAIS/ISS), a student's *t*-test or linear-trend test as well as descriptive statistics were performed using SPSS for Windows; $P \leqslant 0.05$ was determined as the level of significance. Seniors are defined as aged 65 years or older in our study design. The control group to which the results of the senior group were compared was defined as younger than 65 years of age.

RESULTS

From 1999 to 2011, we collected 4430 people aged 65 years or older involved in traffic accidents from a total of 46 490 traffic accident victims.

Demographic data

A total of 2454 were male seniors (55.4%). There were 1928 car drivers, 896 car riders and 46 truck drivers. 828 drove two-wheel motorized vehicles or bicycles and 638 seniors were involved in traffic accidents as pedestrians. A total of 2482 seniors were found guilty of being responsible for the accident (56.4%). In the group of people younger than 65 years of age, 50.7% were found guilty of being responsible for the accident.

Medical analysis

The maximum abbreviated injury scale[16] in our group was 6 with 28 seniors, 34 with a MAIS 5, 51 with a MAIS 4, and 4133 with a MAIS 3 or lower (93.2%). Some 119 seniors sustained injuries to the head with at least MAIS 3 or higher (2.7%). Overall, 12 seniors sustained lethal injuries to the head with an AIS 6, 23 seniors an AIS 5, and 4245 an AIS 4 or lower (95.8%). Injuries to the neck and maxillofacial region occurred in 531 victims in our group (12%), AIS 3 or lower in 390, and AIS 4 or higher in 141 cases. With an AIS 4 or higher, 57 seniors in our group sustained severe injuries to the chest, 934 sustained

injuries with an AIS 3 or lower (21%). Moderate to mild abdominal trauma was observed in 138 victims of our group. Severe trauma with a MAIS higher than 3 to the abdominal region was seen in 25 elderly trauma patients.

Pelvic trauma with higher MAIS scores was a rare but observed injury, with 25 patients with an AIS higher than 3. Besides injuries to the head and maxillofacial region, injuries to upper and lower extremities were often observed. Severe injuries to lower extremities with a MAIS 3 or higher were sustained by 131 of the elderly, with comparable injuries to the upper extremities by 30 accident victims. Compared to other groups involved in daily traffic scenes, the prevalence and severity of injuries to head, maxillofacial region and upper or lower extremities were significantly more frequent ($P = 0.002$, students-t-test for independent variables). Secondly, the overall percentage of accident-related injuries was higher in the group of older people (60% *vs* 52% younger than 65 years of age). There were no gender-related differences observed in accident-related injury patterns in the elderly.

Technical crash analysis

Technical in-depth crash investigation was performed in all observed traffic accidents. At a travelling speed of 2-40 km/h, 8.8% of all car passengers sustained moderate to severe injuries (MAIS 2+). With an increase of the travelling speed above 40 km/h, the number of injured passengers sustaining moderate to severe injuries increased to 41.2%. Only 8.1% of all passengers were not injured at this collision speed. There were no substantial differences within the groups of younger or older people.

Different from car passengers, motor bicyclists were injured more severely and more frequently throughout the whole speed range. With closing speeds of up to 20 km/h, 27.3% of all older motor bicyclists sustained severe injuries (19.5% in the group of younger motor bicyclists). With an increase in travelling speed, the proportion of severely injured motor bicyclists increased to 55.6% in the group of older people (27.3% in the younger riders). Comparable trends were observed with bicyclists colliding with cars.

At a collision speed of 21-40 km/h, 46.9% of older bicyclists sustained serious to severe injuries, whereas in the group of younger people, only 30.9% of bicyclists were seriously injured. Within the group of pedestrians, 79.8% of older people sustained severe to serious injuries when colliding with a car travelling at a speed of 21-40 km/h (43.4% younger people). Additionally, 14.3% of injured older pedestrians suffered severe to fatal injuries. Overall analyses of the accident situation showed that seniors are more likely to sustain severe injuries, especially as so-called external or vulnerable road users. The reasons for this can be direct transfer of impact energy and, with this, a disproportionally frequent inclusion of head and thoracic areas due to the impact kinematics.

DISCUSSION

The mean age of the German population is still increasing and so does the number of people aged 65 years or older taking part in daily traffic and maintaining their continuous level of motorization as a part of their independence. So, the number of seniors involved in traffic accidents has grown over the last years (Federal Statistical Office Germany, Report 2010).

The objective of our retrospective study was to analyze the actual traffic injury situation of older people, including MAIS and AIS/ISS, and special injury patterns compared to younger people. The main findings in our analysis were: firstly, a higher prevalence and severity of injuries to head, maxillofacial region and extremities in older people; secondly, older people are overall more likely to sustain injuries in road traffic accidents; and thirdly, there is a higher percentage of the elderly population responsible for road traffic accidents.

Accidents involving older pedestrians, car drivers or riders account for more than two-third of all involved seniors. Injury analysis in our study shows similar results compared to earlier investigations, as seniors seem to be more likely to be involved in road traffic accidents and sustain more severe injuries with minor trauma[1-3,8]. Allard described the specific needs of accident prevention of the elderly living in an inner city. McCoy showed similar results with an increase in morbidity and mortality in the elderly compared to younger traffic participants. However, a quantitative comparison to previous reports was not part of this study. Our results can be seen as a consequence of the physiological changes with increased age, such as decrease in balance, vision, hearing and reaction time. For further improvement and a reduction of severe accidents caused by seniors in traffic accidents, it is important to realize the special environment of older people[7]. Caused by physiological changes as described earlier, the reaction time and the time needed for analyzing recognized situations is often extended. In addition, seniors seemed to be overwhelmed by the amount of information given nowadays in regular traffic situations[5]. The accident causations could not be analyzed within this study and future studies should give special focus to this.

There are already several injury scores and injury observations focusing on older people, particularly considering the different and special circumstances in which older people take part in daily traffic scenes[3,17,18]. Here, we used the AIS scale. This scale is limited to the moment of the accident event and does not include age-related long term effects. Special focus should be given in future studies on better injury scaling related to age.

Prevention as a method of choice[17] with educational programs is a necessary tool in combination with an overall improvement in street safety to decrease the number of seniors in road traffic accidents[19]. Here, only

detailed in-depth studies can show special age-related results, *i.e.* crossing points for the elderly, special accident types and influences on the accident kinematics. Within the presented study, only an overview of the age related casualties should be given. In future studies, physicians should be integrated to ensure the coordination between physiological changes on one hand[20,21], and changes in environmental circumstances and the special needs of seniors[19,22]. Engineers should work out how safety features like warning signals, speed limiters, road stabilizers and infrastructure can be linked together to avoid accidents in the elderly. Medical doctors should work on precision of injury pattern and long term related injury outcomes. Only in an interdisciplinary coordinated study can the most efficient reduction of injury severity be approached.

In conclusion, seniors are more likely to be involved in traffic injuries and to sustain serious to severe injuries compared to other groups. To improve the safety of all road users, special focus should be given to improved car and street designs for more safety and visibility. In addition, educational programs should improve the active safety of older people in traffic.

ACKNOWLEDGMENTS

For the present study, accident data from GIDAS have been used. Use of the data is restricted to the participants of the project. However, to allow interested parties the direct use of the GIDAS data, several models of participation exist.

COMMENTS

Background

Due to the rising mean age of the German population, traffic accidents involving older people are observed more and more often. Furthermore, previous investigations have shown that geriatric trauma patients are more often associated with a higher level of mortality, morbidity and frequency of more severe injuries. The background of this study was to evaluate actual injury patterns in older patients caused by traffic accidents. The study design was retrospective; data were used from the German In-Depth Accident Study (GIDAS). The results were then compared to injury patterns of people younger than 65 years of age.

Research frontiers

Limitations and research frontiers of the presented study are the retrospective design of the study by analyses of accident data, crash analysis at the scene performed by different examiners and the presented results in a global overview manner.

Innovations and breakthroughs

Seniors are more likely to be involved in traffic injuries and to sustain serious to severe injuries compared to other groups. To improve the safety of all road users, special focus should be given to improved car and street designs for more safety and visibility. In addition, educational programs should improve the active safety of older people in traffic.

Applications

The results of this study can lead to improvements in safety features in cars, bikes and on road crossings. Future urban designs should pay attention to the special needs of older people. Additionally, educational programs should improve road safety for all road users.

Terminology

GIDAS is the largest in-depth accident study in Germany. The data collected in the GIDAS project is extensive and serves as a basis of knowledge for different groups of interest. Since mid 1999, the GIDAS project has collected on-scene accident cases in the areas of Hannover and Dresden. GIDAS collects data from accidents of all kinds and, due to the on-scene investigation and the full reconstruction of each accident, gives a comprehensive view on the individual accident sequences and their causes; ISS is a well-known medical score to assess trauma severity; AIS is a severity scoring system classifying injuries in each body region. Classification ranges from minor to maximum (1-6), indicating the relative severity of each body region. MAIS indicates the maximum AIS-Scale of a trauma victim.

Peer review

The purpose of this article was "to evaluate the overall outcome of older people involved in traffic accidents as a retrospective study from the Hannover in-depth accident research unit. Points of interest were morbidity, mortality and special injury patterns in seniors (65 years+) compared to younger people". This is a well-written paper.

REFERENCES

1 **Allard R**. Excess mortality from traffic accidents among elderly pedestrians living in the inner city. *Am J Public Health* 1982; **72**: 853-854
2 **Brorsson B**. [Elderly drivers run a greater risk than middle-aged drivers of being involved in accidents]. *Lakartidningen* 1989; **86**: 1915-1917
3 **McCoy GF**, Johnston RA, Duthie RB. Injury to the elderly in road traffic accidents. *J Trauma* 1989; **29**: 494-497
4 **Perdue PW**, Watts DD, Kaufmann CR, Trask AL. Differences in mortality between elderly and younger adult trauma patients: geriatric status increases risk of delayed death. *J Trauma* 1998; **45**: 805-810
5 **Abou-Raya S**, ElMeguid LA. Road traffic accidents and the elderly. *Geriatr Gerontol Int* 2009; **9**: 290-297
6 **Ekman R**, Welander G, Svanström L, Schelp L, Santesson P. Bicycle-related injuries among the elderly--a new epidemic? *Public Health* 2001; **115**: 38-43
7 **Bilban M**. Road traffic accidents caused by elderly drivers. *Coll Antropol* 1997; **21**: 573-583
8 **Gebska-Kuczerowska A**, Miller M, Wysocki M, Supranowicz P, Rabczenko D. [Assessment of the determinants of accidents and injuries among elderly people]. *Wiad Lek* 2002; **55** Suppl 1: 91-96
9 **Kazár G**, Bauer O, Kósa J, Pestessy J. [Accidents of the elderly]. *Orv Hetil* 1996; **137**: 1245-1249
10 **Gresset JA**, Meyer FM. Risk of accidents among elderly car drivers with visual acuity equal to 6/12 or 6/15 and lack of binocular vision. *Ophthalmic Physiol Opt* 1994; **14**: 33-37
11 **Ysander L**. Sick and handicapped drivers. A study on the risks of sudden illness at the wheel and on the frequency of road accidents and traffic offences in chronically sick, disabled, and elderly drivers. *Acta Chir Scand Suppl* 1969; **409**: 1-82
12 **Ross N**, Timberlake GA, Rubino LJ, Kerstein MD. High cost of trauma care in the elderly. *South Med J* 1989; **82**: 857-859
13 **Wong TW**. Road traffic accidents in the elderly--a major cause of morbidity and mortality. *Ann Acad Med Singapore* 1987; **16**: 101-105
14 **Osler T**, Hales K, Baack B, Bear K, Hsi K, Pathak D, Demarest G. Trauma in the elderly. *Am J Surg* 1988; **156**: 537-543
15 **Silveira R**, Rodrigues RA, da Costa Júnior ML. [Elderly people victims of traffic accidents in the municipality of Ribeirao Preto-SP, in 1998]. *Rev Lat Am Enfermagem* 2002; **10**: 765-771
16 **Haasper C**, Junge M, Ernstberger A, Brehme H, Hannawald L, Langer C, Nehmzow J, Otte D, Sander U, Krettek C, Zwipp H. [The Abbreviated Injury Scale (AIS). Options and problems in application]. *Unfallchirurg* 2010; **113**: 366-372
17 **Ytterstad B**. The Harstad injury prevention study: community based prevention of fall-fractures in the elderly evaluated by means of a hospital based injury recording system in Norway. *J Epidemiol Community Health* 1996; **50**: 551-558
18 **Vorko-Jović A**, Jović F. Macro model prediction of elderly

people's injury and death in road traffic accidents in Croatia. *Accid Anal Prev* 1992; **24**: 667-672
19 **Hansen TB**, Dupont G. [Fatal traffic accidents among the elderly in the county of Ringkøbing]. *Ugeskr Laeger* 1992; **154**: 3676-3678
20 **Herner B**. [Physician's role in the prevention of traffic accidents among elderly drivers is of significance]. *Lakartidningen* 1991; **88**: 1172
21 **Snipes GE**. Accidents in the elderly. *Am Fam Physician* 1982; **26**: 117-122
22 **Michiels W**, Fryc O, Krompecher T. [Responsibility in the prevention of accidents of the most exposed pedestrians: children and elderly persons]. *Soz Praventivmed* 1982; **27**: 26-29

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INSTRUCTIONS TO AUTHORS

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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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Conference paper

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Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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