**Supplemental Information for**

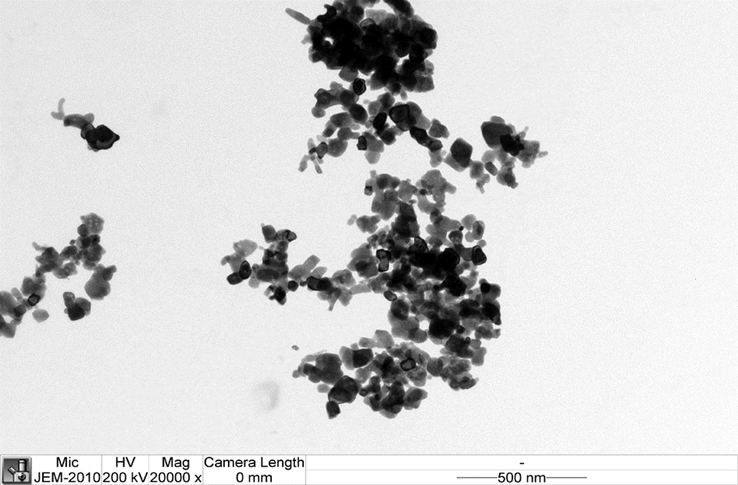
The Early Onset and Persistent Worsening Pulmonary Alveolar Proteinosis in Rats by Indium Oxide Nanoparticles

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**Figure S1** The size and morphology evaluated by the transmission electron microscopy.



**Figure S2** The time-course inflammation pattern evaluated by the percentage of inflammatory cells in bronchoalveolar lavage fluid after a single instillation of In2O3 NPs. A, The percentage of macrophages; B, the percentage of neutrophils; C, the percentage of lymphocytes. Data are presented as mean ± SEM. *n*=8 for each group at 1-30 days and *n*=4 for each group at 90-180 days. VEH, vehicle control.

G:\00-Indium Lung inflammation\PFT\Final-Low dose-small size.tif

**Figure S3** The time-course colour changes of bronchoalveolar lavage fluid after single instillation of In2O3 NPs. Cell-free bronchoalveolar lavage fluid was transferred to cuvette cells and pictures were taken with digital camera. VEH, vehicle control.

**Table S1.** The numeric data with statistical analysis for Figure 1.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Time point | 1 | 3 | 7 | 14 | 30 | 90 | 180 |
| Number of total cells (×106 cells) | | | | | | | |
| * VEH | 4.44  (0.364) | 3.07  (0.738) | 3.49  (0.760) | 2.96  (0.432) | 5.05  (0.890) | 9.60  (1.015) | 4.68  (0.295) |
| * Low | 3.52  (0.361) | 4.01  (0.833) | 5.34  (0.901) | 9.99  (1.190) | 13.58\*  (1.593) | 16.01  (1.313) | 10.46\*  (0.984) |
| * Mid | 6.45  (0.957) | 6.50  (0.897) | 11.09\*  (1.907) | 16.52\*\*\*  (1.479) | 25.29\*\*\*  (2.834) | 20.49  (2.959) | 11.82\*\*  (1.109) |
| * High | 7.47  (0.700) | 7.83\*  (0.987) | 11.73\*  (1.116) | 20.04\*\*\*  (2.748) | 22.89\*\*\*  (2.495) | 28.70\*\*  (4.791) | 15.24\*\*\*  (2.011) |
| Number of macrophages (×106 cells) | | | | | | | |
| * VEH | 4.34  (0.372) | 3.07  (0.738) | 3.49  (0.760) | 2.96  (0.432) | 5.03  (0.876) | 9.49  (1.000) | 4.680  (0.295) |
| * Low | 3.09  (0.312) | 2.92  (0.456) | 3.62  (0.559) | 5.88  (0.974) | 9.08  (0.977) | 11.34  (1.151) | 7.52\*  (0.461) |
| * Mid | 2.50\*\*  (0.144) | 2.79  (0.353) | 3.41  (0.841) | 7.44\*\*  (1.000) | 12.03  (1.944) | 7.44  (0.651) | 7.74\*  (1.142) |
| * High | 2.99  (0.387) | 2.92  (0.351) | 3.13  (0.440) | 5.14  (0.712) | 11.23\*  (1.779) | 10.54  (1.909) | 5.93  (0.252) |
| Number of neutrophils (×106 cells) | | | | | | | |
| * VEH | 0.10  (0.025) | 0  (0) | 0  (0) | 0  (0) | 0.02  (0.015) | 0.06  (0.034) | 0  (0) |
| * Low | 0.409  (0.124) | 1.09  (0.408) | 1.69  (0.397) | 4.07  (0.551) | 4.26  (0.832) | 4.33  (0.390) | 2.84  (0.481) |
| * Mid | 3.92\*  (0.866) | 3.62\*\*  (0.543) | 7.64\*\*\*  (1.327) | 9.04\*\*\*  (0.874) | 12.94\*\*\*  (1.709) | 12.69\*\*  (2.265) | 4.02  (0.208) |
| * High | 4.45\*\*  (0.802) | 4.88\*\*\*  (0.717) | 8.47\*\*\*  (0.832) | 14.85\*\*\*  (2.049) | 11.33\*\*\*  (1.702) | 17.52\*\*\*  (2.779) | 9.09\*\*\*  (1.935) |
| Number of lymphocytes (×106 cells) | | | | | | | |
| * VEH | 0  (0) | 0  (0) | 0  (0) | 0  (0) | 0  (0) | 0.032  (0.019) | 0  (0) |
| * Low | 0  (0) | 0  (0) | 0.012  (0.004) | 0.028  (0.012) | 0.240  (0.075) | 0.330\*  (0.041) | 0.095  (0.043) |
| * Mid | 0.002  (0.002) | 0.002  (0.002) | 0.040  (0.014) | 0.044  (0.017) | 0.319\*  (0.095) | 0.358\*  (0.061) | 0.062  (0.028) |
| * High | 0  (0) | 0  (0) | 0.128\*  (0.039) | 0.054  (0.019) | 0.314\*  (0.043) | 0.634\*\*\*  (0.111) | 0.222\*\*  (0.038) |

Data are presented as mean (SEM).

Each treatment group was compared with the vehicle control (VEH) to determine statistical significance (One-way analysis of variance (ANOVA), followed by *post hoc* Tukey’s pairwise comparison). \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001.

**Table S2**. The numeric data with statistical analysis for Figure S2.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Time point | 1 | 3 | 7 | 14 | 30 | 90 | 180 |
| Percentage of macrophages | | | | | | | |
| * VEH | 97.1  (0.78) | 100  (0) | 100  (0) | 100  (0) | 99.7  (0.15) | 98.8  (0.28) | 100  (0) |
| * Low | 88.2  (2.70) | 78.5\*\*  (4.31) | 69.6\*\*\*  (3.12) | 58.0\*\*\*  (4.36) | 68.1\*\*\*  (3.60) | 70.6\*\*\*  (2.41) | 72.5\*\*\*  (2.07) |
| * Mid | 42.2\*\*\*  (3.90) | 43.7\*\*\*  (2.29) | 30.2\*\*\*  (4.23) | 44.6\*\*\*  (3.80) | 48.3\*\*\*  (5.53) | 37.5\*\*\*  (2.68) | 64.4\*\*\*  (4.22) |
| * High | 41.8\*\*\*  (5.69) | 38.0\*\*\*  (2.32) | 26.3\*\*\*  (2.21) | 25.8\*\*\*  (0.73) | 48.7\*\*\*  (4.23) | 36.5\*\*\*  (0.66) | 40.4\*\*\*  (3.95) |
| Percentage of neutrophils | | | | | | | |
| * VEH | 2.8  (0.73) | 0  (0) | 0  (0) | 0  (0) | 0.2  (0.14) | 0.57  (0.34) | 0  (0) |
| * Low | 11.2  (2.68) | 21.5\*\*\*  (4.32) | 29.8\*\*\*  (3.10) | 41.7\*\*\*  (4.42) | 30.3\*\*\*  (3.56) | 27.2\*\*\*  (2.55) | 26.7\*\*\*  (1.79) |
| * Mid | 57.3\*\*\*  (3.90) | 55.3\*\*\*  (2.31) | 69.2\*\*\*  (4.14) | 55.1\*\*\*  (3.70) | 50.5\*\*\*  (5.56) | 60.8\*\*\*  (2.65) | 35.2\*\*\*  (4.29) |
| * High | 57.8\*\*\*  (5.80) | 61.8\*\*\*  (2.30) | 72.5\*\*\*  (2.11) | 73.9\*\*\*  (0.77) | 49.8\*\*\*  (4.35) | 61.3\*\*\*  (0.66) | 58.0\*\*\*  (4.23) |
| Percentage of lymphocytes | | | | | | | |
| * VEH | 0  (0) | 0  (0) | 0  (0) | 0  (0) | 0  (0) | 0.33  (0.19) | 0  (0) |
| * Low | 0  (0) | 0  (0) | 0.30  (0.12) | 0.23  (0.10) | 1.60\*\*\*  (0.35) | 2.07\*\*\*  (0.24) | 0.83  (0.29) |
| * Mid | 0.04  (0.04) | 0.04  (0.04) | 0.46  (0.14) | 0.31  (0.13) | 1.14\*  (0.25) | 1.73\*\*\*  (0.07) | 0.49  (0.22) |
| * High | 0  (0) | 0  (0) | 1.17\*\*  (0.39) | 0.32  (0.12) | 1.49\*  (0.26) | 2.21\*\*\*  (0.08) | 1.59\*\*  (0.44) |

Data are presented as mean (SEM).

Each treatment group was compared with the vehicle control (VEH) to determine statistical significance (One-way analysis of variance (ANOVA), followed by *post hoc* Tukey’s pairwise comparison). \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001.

**Table S3**. The numeric data with statistical analysis for Figures 2 and 3.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Time point | 1 | 3 | 7 | 14 | 30 | 90 | 180 |
| LDH (fold vs VEH) | | | | | | | |
| * VEH | 1.00  (0.067) | 1.00  (0.089) | 1.00  (0.001) | 1.00  (0.095) | 1.00  (0.156) | 1.00  (0.211) | 1.00  (0.095) |
| * Low | 1.40  (0.372) | 1.39  (0.066) | 2.14  (0.366) | 6.46\*\*\*  (0.339) | 4.83  (1.077) | 4.31\*  (0.277) | 5.54  (0.505) |
| * Mid | 1.91\*  (0.105) | 4.95\*\*\*  (0.899) | 7.22\*\*\*  (0.573 | 9.66\*\*\*  (0.640) | 8.57\*\*  (0.945) | 11.78\*\*\*  (0.994) | 15.41\*\*\*  (0.323) |
| * High | 2.75\*\*\*  (0.141) | 7.94\*\*\*  (0.437) | 8.97\*\*\*  (0.449) | 13.96\*\*\*  (1.210) | 15.43\*\*\*  (1.293) | 16.98\*\*\*  (0.801) | 20.45\*\*\*  (2.503) |
| Total protein (μg/mL) | | | | | | | |
| * VEH | 112.9  (9.14) | 131.3  (11.83) | 131.3  (11.83) | 105.9  (7.81) | 109.5  (46.23) | 170.8  (28.20) | 147.1  (11.46) |
| * Low | 170.8  (25.38) | 201.6  (8.99) | 240.1  (32.38) | 620.0\*\*\*  (21.28) | 549.5  (182.4) | 595.4\*  (56.67) | 634.3  (72.24) |
| * Mid | 193.8  (15.75) | 486.8\*\*\*  (71.57) | 682.4\*\*\*  (59.42) | 942.0\*\*\*  (52.32) | 1253\*\*  (228.6) | 1467\*\*\*  (88.94) | 1775\*\*\*  (67.92) |
| * High | 275.8\*\*  (36.33) | 651.6\*\*\*  (41.58) | 877.9\*\*\*  (20.41) | 1335\*\*\*  (64.40) | 1502\*\*\*  (141.3) | 2210\*\*\*  (119.6) | 2955\*\*\*  (302.7) |
| Phospholipids (μM) | | | | | | | |
| * VEH | 40.32  (4.93) | 39.70  (5.05) | 41.83  (6.63) | 41.01  (6.90) | 45.24  (9.45) | 146.4  (7.804) | 275.8  (42.49) |
| * Low | 53.20  (17.01) | 46.84  (8.36) | 116.0  (34.10) | 1359  (84.56) | 831.2  (316.4) | 1117  (124.2) | 1128  (230.4) |
| * Mid | 102.2  (13.39) | 513.0\*\*  (120.4) | 1131\*\*\*  (93.49) | 2951\*\*  (754.5) | 7631\*\*\*  (869.5) | 3892\*\*  (879.3) | 5115\*\*  (1833) |
| * High | 224.6\*\*\*  (19.70) | 987.8\*\*\*  (101.5) | 1328\*\*\*  (153.2) | 3921\*\*\*  (376.8) | 5778\*\*\*  (888.8) | 4029\*\*  (613.7) | 5448\*\*\*  (765.0) |
| Turbidity (OD600) | | | | | | | |
| * VEH | 0.054  (0.0027) | 0.052  (0.0006) | 0.055  (0.0018) | 0.054  (0.0028) | 0.053  (0.0009) | 0.053  (0.0009) | 0.049  (0.0013) |
| * Low | 0.063  (0.0046) | 0.061  (0.0057) | 0.110\*  (0.0121) | 0.307\*\*  (0.0518) | 0.229  (0.0406) | 0.100  (0.0139) | 0.147  (0.0681) |
| * Mid | 0.0565  (0.0033) | 0.096\*\*  (0.0104) | 0.213\*\*\*  (0.0147) | 0.431\*\*\*  (0.0475) | 0.958\*\*\*  (0.0920) | 0.271\*\*  (0.0197) | 0.626\*\*  (0.0390) |
| * High | 0.0585  (0.0035) | 0.098\*\*  (0.0107) | 0.239\*\*\*  (0.0148) | 0.282\*\*  (0.0271) | 0.790\*\*\*  (0.1020) | 0.666\*\*\*  (0.0609) | 0.893\*\*\*  (0.1670) |

Data are presented as mean (SEM).

Each treatment group was compared with the vehicle control (VEH) to determine statistical significance (One-way analysis of variance (ANOVA), followed by *post hoc* Tukey’s pairwise comparison). \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001.

**Table S4**. The results of statistical analysis for Figure 4.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Time point | 1 | 3 | 7 | 14 | 30 | 90 | 180 |
| IL-1β (pg/mL) | | | | | | | |
| * VEH | 1.54  (1.54) | 0  (0) | 0  (0) | 0  (0) | 0  (0) | 1.54  (1.54) | 25.39  (8.68) |
| * Low | 96.59\*  (34.77) | 43.01  (8.74) | 30.32  (15.19) | 82.98  (5.59) | 40.32  (12.67) | 20.66  (8.63) | 61.92  (11.74) |
| * Mid | 144.8\*\*  (8.43) | 105.0\*\*  (32.16) | 122.8  (9.42) | 138.3\*  (35.14) | 539.7\*\*  (102.2) | 164.1  (39.64) | 190.9  (25.22) |
| * High | 141.4\*\*  (14.60) | 109.8\*\*  (9.57) | 197.6\*\*  (67.51) | 135.4\*  (50.58) | 351.7\*  (112.6) | 357.7\*\*  (94.16) | 309.9\*\*  (86.99) |
| TNF-α (pg/mL) | | | | | | | |
| * VEH | 0  (0) | 5.60  (2.82) | 0  (0) | 0  (0) | 0  (0) | 0  (0) | 0  (0) |
| * Low | 17.44  (8.75) | 3.77  (2.41) | 3.14  (3.14) | 3.47  (2.02) | 3.31  (1.72) | 3.01  (2.78) | 1.96  (1.15) |
| * Mid | 5.98  (2.32) | 9.03  (7.46) | 9.99  (3.82) | 13.02  (4.42) | 27.35\*\*  (4.90) | 16.99  (3.00) | 17.19\*\*  (3.27) |
| * High | 10.03  (3.39) | 15.36  (3.34) | 15.28\*  (5.22) | 32.10\*  (12.22) | 32.97\*\*\*  (5.77) | 29.18\*\*  (7.55) | 38.14\*\*\*  (4.88) |
| MCP-1 (pg/mL) | | | | | | | |
| * VEH | 0  (0) | 0.03  (0.03) | 0.06  (0.06) | 0  (0) | 0  (0) | 0.09  (0.06) | 0.09  (0.06) |
| * Low | 0.02  (0.02) | 0  (0) | 1.79  (0.96) | 56.36  (6.76) | 52.21  (27.46) | 54.11\*\*\*  (5.18) | 38.64  (10.39) |
| * Mid | 1.72  (0.91) | 11.59\*  (4.78) | 44.42\*  (15.36) | 162.9\*\*\*  (3.23) | 166.8\*\*\*  (5.42) | 160.0\*\*\*  (9.25) | 149.8\*\*\*  (5.72) |
| * High | 3.89\*\*  (0.91) | 9.20  (2.62) | 51.63\*  (12.11) | 138.7\*\*\*  (34.92) | 174.4\*\*\*  (1.94) | 176.9\*\*\*  (3.20) | 157.4\*\*\*  (16.81) |
| CINC-3 (pg/mL) | | | | | | | |
| * VEH | 0  (0) | 0  (0) | 0  (0) | 0  (0) | 0  (0) | 0  (0) | 0  (0) |
| * Low | 58.73  (12.94) | 15.42  (2.94) | 28.62  (14.06) | 29.98\*  (5.16) | 7.29  (3.40) | 3.34  (3.34) | 2.83  (2.83) |
| * Mid | 200.4\*\*\*  (38.17) | 19.77\*  (4.19) | 45.95  (26.24) | 14.49  (6.19) | 2.96  (2.96) | 1.40  (1.40) | 18.90  (9.38) |
| * High | 72.21  (34.57) | 35.53\*\*\*  (7.63) | 27.35  (7.52) | 8.40  (3.96) | 14.69  (5.87) | 8.72  (4.47) | 2.39  (2.39) |

Data are presented as mean (SEM).

Each treatment group was compared with the vehicle control (VEH) to determine statistical significance (One-way analysis of variance (ANOVA), followed by *post hoc* Tukey’s pairwise comparison). \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001.

**Table S5.** The hematological analysis at 3 and 6 months after single instillation of In2O3 NPs to rats.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Time-points | At 3 months | | | |  | At 6 months | | | |
| Dose | VEH | Low | Mid | High |  | VEH | Low | Mid | High |
| RBC (×106 cells/μL) | 6.82 ± 0.89 | 7.61 ± 0.17 | 5.24 ± 0.96 | 7.33 ± 0.14 |  | 7.96 ± 0.17 | 7.69 ± 0.14 | 7.78 ± 0.09 | 7.52 ± 0.23 |
| HGB (g/dL) | 14.6 ± 0.31 | 14.5 ± 0.24 | 14.1 ± 0.46 | 15.0 ± 0.10 |  | 14.6 ± 0.18 | 13.9 ± 0.14 | 14.1 ± 0.14 | 14.2 ± 0.28 |
| HCT (%) | 45.18 ± 5.23 | 49.28 ± 1.55 | 36.4 ± 6.39 | 48.9 ± 0.65 |  | 45.5 ± 0.55 | 44.4 ± 0.39 | 45.2 ± 0.51 | 45.2 ± 0.81 |
| MCV (fL) | 66.65 ± 1.21 | 64.78 ± 0.78 | 70.15 ± 2.13 | 66.66 ± 0.74 |  | 57.2 ± 0.76 | 57.7 ± 0.57 | 58.1 ± 1.05 | 60.3 ± 1.04 |
| MCHC (g/dL) | 33.75 ± 4.30 | 29.58 ± 1.14 | 42.95 ± 8.25 | 30.82 ± 0.46 |  | 32.16 ± 0.14 | 31.32 ± 0.30 | 31.24 ± 0.22 | 31.48 ± 0.45 |
| PLT (×103 cells/μL) | 1063 ± 173 | 728 ± 34 | 1289 ± 131 | 1037 ± 62 |  | 858 ± 53 | 828 ± 31 | 794 ± 76 | 888 ± 57 |
| WBC (×103 cells/μL) | 2.84 ± 0.24 | 2.23 ± 0.21 | 2.67 ± 0.30 | 3.17 ± 0.33 |  | 2.51 ± 0.24 | 2.75 ± 0.27 | 2.84 ± 0.18 | 3.88 ± 0.79 |
| Neutrophils (×103 cells/μL) | 0.31 ± 0.05 | 0.37 ± 0.04 | 0.64 ± 0.05 | 0.86 ± 0.09 |  | 0.42 ± 0.04 | 0.59 ± 0.17 | 0.56 ± 0.02 | 1.30 ± 0.53 |
| Lymphocytes (×103 cells/μL) | 2.40 ± 0.17 | 1.90 ± 0.21 | 1.88 ± 0.25 | 2.19 ± 0.24 |  | 1.96 ± 0.21 | 2.03 ± 0.10 | 2.16 ± 0.17 | 2.36 ± 0.28 |
| Monocytes (×103 cells/μL) | 0.02 ± 0.01 | 0.02 ± 0.02 | 0.04 ± 0.01 | 0.03 ± 0.01 |  | 0.04 ± 0.01 | 0.06 ± 0.01 | 0.04 ± 0.00 | 0.09 ± 0.03 |
| Eosinophils (×103 cells/μL) | 0.09 ± 0.04 | 0.04 ± 0.01 | 0.06 ± 0.01 | 0.07 ± 0.02 |  | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.07 ± 0.01 | 0.11 ± 0.04 |
| Basophils (×103 cells/μL) | 0.02 ± 0.01 | 0.00 ± 0.00 | 0.03 ± 0.01 | 0.02 ± 0.00 |  | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.04 ± 0.02 |

Note that there were no statistically significant changes in any treatment groups compared to vehicle control. Data are expressed as mean ± SEM and *n*=5.